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Contents

The Proper Supplementary Ration for Milking Cows on Fertilized Pasture. RICHARD C. FOLEY 407

Dietary Acidosis in Dairy Cattle. A. E. PERKINS and C. F. MONROE 418

The Effects of Some Fly Sprays on the Hides and on the Body Temperatures of Dairy Cows. JOHN L. WILSON, A. M. PEARSON, and C. Y. CANNON 427

The Effect of Pasteurizing on the Bacterial Flora of the Ice Cream Mix. CHARLES M. DUBOIS and W. H. MARTIN 435

The Phospholipids in Milk:

I. *Their Distribution Among Some Milk Products.* GEORGE E. HOLM, PHILIP R. WRIGHT, and EDGAR F. DEYSHER 445

II. *The Phospholipids in Skimmilks and Their Effect Upon the Accuracy of the Various Fat Tests.* PHILIP A. WRIGHT and GEORGE E. HOLM 455

III. *The Phospholipids in Buttermilk and Their Effect Upon the Accuracy of Various Fat Tests.* PHILIP A. WRIGHT, EDGAR F. DEYSHER, and GEORGE E. HOLM 460

Factors Governing the Manufacture of Sweet-Curd Cottage Cheese. L. M. THURSTON and IRA GOULD, JR. 467

The Escherichia-Aerobacter Group of Bacteria in Dairy Products. M. W. YALE 481

The Preservation of Skimmilk Curd for Poultry Feeding. R. N. DAVIS 495

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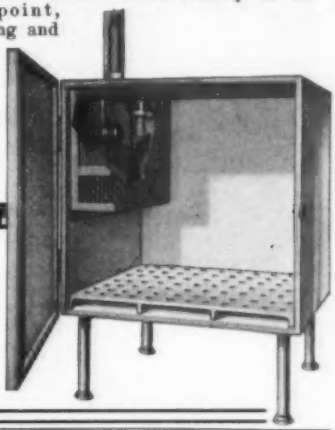

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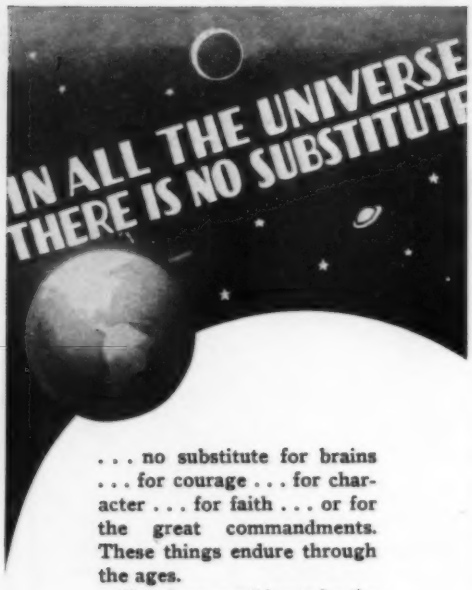

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JOURNAL OF DAIRY SCIENCE

VOLUME XVI SEPTEMBER, 1933 NUMBER 5

THE PROPER SUPPLEMENTARY RATION FOR MILKING COWS ON FERTILIZED PASTURE[1]

RICHARD C. FOLEY[2]

Massachusetts Agricultural Experiment Station, Amherst

In the beginning, nature endowed the progenitors of our modern dairy cow with the ability to produce sufficient milk to nourish their calves until those youngsters were able to rustle for themselves. She also furnished these early cattle with an abundance of lush spring grass during the lactation period. As the season advanced the grass contained less protein and more carbohydrate and fiber. The cows gradually dried off and began to restore the flesh lost while nursing their calves.

Through the years, by selection and feeding, we have extended the lactation period to a full year and increased the yield of milk many times, supplying the necessary additional nutrients largely in the form of the concentrated by-products of cereal grains. Until recently, we had made no serious attempt to lengthen the productive period or to improve the yield and quality of nature's own feed for the dairy cow, namely, pasture grass. The recent low price of milk, however, has centered attention upon most economical rather than maximum production, and the present low cost of fertilizer materials and new developments in pasture management have pointed the way to this goal.

This study in economy of production also presented an opportunity to test another hypothesis; namely that some dry roughage throughout the season is a desirable addition to the ration of milch cows on fertilized pasture. Observations on the Massachusetts State College farm and recent reports from England indicated that cows relish mixed hay, straw or other dry roughage in addition to pasture. The inroads made on straw stacks in corn belt feed lots by beef steers on full feed likewise portray nature's attempt to balance the ration. Finally, it has been frequently noted that cattle tend to browse more on fertilized pasture.

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[1] Massachusetts Agricultural Experiment Station Contribution No. 155, 1932.

[2] The author wishes to express to Prof. C. H. Parsons, Farm Superintendent, and Thomas Muir, Herdsman, his appreciation of their assistance in carrying on this trial, and to acknowledge the cooperation of the Experiment Station which made this trial possible.

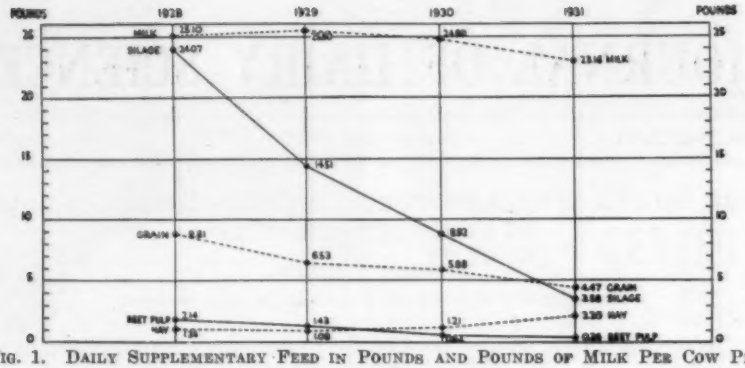

FIG. 1. DAILY SUPPLEMENTARY FEED IN POUNDS AND POUNDS OF MILK PER COW PER DAY 1928–1931

Figure 1 illustrates the success with which supplementary barn feeding during the summer months has been reduced through the application of intensive grassland management on the college farm at the Massachusetts State College. Silage and beet pulp have been practically eliminated from the summer feeding program on the college farm. In 1930 hay was fed only while the cattle were being accustomed to pasture in the spring and after being taken off night pasture in the fall. The problem, then, concerned itself with reducing or replacing the concentrates fed in the barn. The protein content had already been lowered from 18 per cent to 14 per cent on the average in accordance with the improved pasture available. Since the college farm produces a surplus of good mixed hay, this roughage immediately suggested itself as a possible substitute for part of the concentrates in the ration. In 1932, in order to meet the problem of many dairymen in the eastern part of the state where the hay crop is short but where oat feed is available, sugared oat feed was fed as a hay substitute to one group.

METHOD OF PROCEDURE

In 1931, two groups of nine cows each were experimentally fed for 100 days. Group A received concentrates only, at the rate of one pound of grain to four and one half pounds of milk, and all the good pasture they would consume. Group B received pasture, all the roughage they would clean up twice a day, and concentrates at the rate of one pound of grain to ten pounds of milk. In 1932 the trial was repeated using three groups of six cows each. Groups A and B were fed the same as in 1931 while Group C received three pounds of oat feed daily in place of the mixed hay fed to Group B.

The amount fed in the barn was kept constant throughout the trial. It was felt that the decreased quality of the pasture as the season advanced

and the influence of advancing lactation would warrant this even though the average daily milk yield was lower. The grain allowance for all cows was sufficiently limited to insure maximum use of pasture. All three groups were pastured together in rotation over the plots of the Hohenheim system and except during the latter part of the 1932 trial had an abundance of good grass at all times. One seeding of oats and one 4 acre plot in Japanese millet and Sudan grass were included with the grass areas. The cattle were weighed at weekly intervals and barn feed was weighed daily.

Table 1 presents the data on the animals used in both trials. Groups A and B are averaged for the two seasons.

TABLE 1

Data on groups fed in trial

	GROUP A	GROUP B	GROUP C
Ration	Pasture Grain —	Pasture Grain Hay	Pasture Grain Oat Feed
Number of Cows	15	15	6
Average age of cows—years and months	4–7	4–5	4–2
Average weight at beginning of trial, pounds	1179	1150	1146
Average days in milk at start of trial	165	160	107
Average days in calf	73	110	55
Average daily production at start of trial	29.5	28.8	31.7
Average butterfat test, per cent	4.7	4.9	4.6
Average daily grain, pounds	5.7	3.1	3.8
Average daily roughage, pounds	None	2.7	3.0

It will be noted in studying table 1 that the oat feed group has an advantage in lactation, gestation, and daily production at the start of the trial as compared with the averaged figure including 1931 and 1932 given for the other two groups. This is due to the fact that all groups averaged fewer days in lactation and gestation and were milking more heavily at the start of the test the second year. Otherwise, the table shows the groups to have been quite evenly balanced as regards those characteristics which would exert an influence on the results of the trial.

For the benefit of those interested in the practical applications of this trial, and to emphasize the fact that on fertilized pasture, a relatively low-protein grain ration can be successfully used, the mixture fed during the two seasons are presented in table 2.

Due to a lack of rainfall, the quality of the pasture during the summer of 1932 was below that of the previous season. Therefore, the protein content of the supplementary grain ration was increased slightly the second year. The proportion of oat feed in the basic ration was reduced from 500 to 300 pounds since one group was to receive it as a substitute for mixed

TABLE 2

Grain mixtures used in trials

	1931			1932		
	June	July	August	June	July	August
Crude Protein, per cent	10	12	15	11	13	16
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Hominy	1000	700	800	1000	1000	500
Gr. Oats	500	300	——	500	400	300
Oat Feed	500	500	500	300	300	300
20% Ration	——	500	——	——	——	400
Wh. Bran	——	——	400	100	100	200
Soy-Bean Meal	——	——	——	100	200	200
Cottonseed-Meal	——	——	300	——	——	100
	2000	2000	2000	2000	2000	2000

hay. Sugared oat feed was used because of its greater palatability and Soy-Bean Meal replaced Linseed-Oil Meal because of its lower unit cost.

RESULTS OBTAINED

Following the plan begun in table 1, the results of the two years' work are summarized in table 3 with Groups A and B averaged for the two seasons. The figures under Group C are for 1932 only.

TABLE 3

Summary of results

	GROUP A	GROUP B	GROUP C
Ration	Grain	Hay	Oat Feed
Length of trial, days	190	190	90
Average gain or loss in weight, pounds	+27	+20	−12
Average daily milk yield, pounds	25.0	24.0	26.4
Yield Sept. 1 in % of initial yield	68	68	64
Average daily grain, pounds	5.7	3.1	3.8
Average daily roughage, pounds	None	2.7	3.0
Grain to milk ratio	1–4.45	1–8.24	1–6.90
Total barn feed cost	$47.22	$38.35	$35.37
Barn feed cost per 100 lbs. milk, cents	27	22	25

In studying table 3 the following points are worthy of notice. While the averages indicate that Group A made the largest gain in weight and Group C showed a slight average loss, all animals in the trial finished the season in good milking condition. The percentage of initial yield is a fairer measure of the production of the groups than are the figures for daily milk yield since it was impractical to have all three groups on exactly the same level at the start of the trial. Thus the animals receiving the roughage

were equally persistent with the check group getting grain at the ratio of one to four. Group B averaged better than 8 pounds of milk per pound of grain fed for the two seasons. This is for milk testing better than 4 per cent butterfat, and the group included representatives of all four dairy breeds and milking Shorthorns. While it is always debatable whether dollars and cents comparisons should be presented in experimental work, in order to arrive at a common basis for comparing the three groups it is necessary to figure feed cost. In the above table, grain was charged at $24.00, while hay and oat feed were figured at $13.00 per ton.

The extremely low barn feed cost per 100 pounds of milk, namely, 22 cents in the hay group is the outstanding result of the two years' work. It indicates that there are still opportunities for considerable reductions in the barn feed cost of milk production through wider untilization of home-grown feed. Results in other herds will vary with the producing ability of the cattle used and the quality of the pasture available but the results of this trial open up new methods of approach to the summer feeding problem.

CONCLUSIONS

1. Two seasons' work indicates that mixed hay is a desirable addition to the ration of milking cows on fertilized pastures.

2. Maximum utilization of home-grown feeds, *i.e.*, good pasture and mixed hay, will result in a wider grain to milk ratio and in lowered cost of milk production during the summer months.

3. Oat feed can probably replace mixed hay when the latter is wanting, or high in price.

4. An abundance of palatable and nutritious pasture throughout the summer is necessary for the successful application of this method of feeding.

DIETARY ACIDOSIS IN DAIRY CATTLE

A. E. PERKINS AND C. F. MONROE
Ohio Agricultural Experiment Station, Wooster, Ohio

INTRODUCTION AND THEORETICAL

Van Slyke (14) has broadly defined "Acidosis" as a condition caused by the formation or ingestion of acids at a rate exceeding that of their elimination. This process may be considered to have caused an abnormal state when it has either increased the hydrion concentration of the blood, or lowered its alkali reserve beyond the extreme normal limit.

Nature has provided several safeguards to maintain the reaction of the blood and through it of the various body tissues at a constant or nearly constant level, and it is important that this is true for a departure of more than 1/10 of a pH value in either direction from the normal of 7.3 to 7.5 in humans is accompanied by distressing symptoms and a departure of 2/10 of a pH value places the subject in danger of tetany on one hand, and of coma on the other.

If an excess of basic materials occurs, the excess is normally combined with carbonic acid to form bicarbonates, which are less basic in character. If an excess of acid occurs it is ordinarily combined with the base portion of the bicarbonate, setting free water and CO_2, which is readily eliminated. The alkali phosphates, and to a lesser extent, the proteins also have the ability to combine with any excess of either acid or base which may occur and thus assist in preserving the proper reaction of the blood stream and body tissues. Ammonia, which is derived from the metabolism of excess protein materials, which are normally excreted as urea, is also frequently used to combine with excess acids.

The acids thus neutralized are normally excreted promptly in the urine. In diseases such as diabetes and nephritis these normal regulatory devices break down and the control of the accompanying acidosis becomes a major problem. Acidosis also occurs as an accompaniment to numerous other diseases.

Acidosis may also be brought about by the use of diets unbalanced with respect to mineral content, as the salt-free diet sometimes used, or by unduly increasing the fats and reducing the carbohydrates, the so-called Ketogenic diet. Acidosis produced by these methods is being introduced in modern human medicine to relieve certain troubles such as bacterial invasion of the urinary tract and as an aid in fighting certain forms of tuberculosis and skin lesions (5), (7). It has also been used in the treatment of epilepsy.

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However useful such controlled acidosis may be under proper conditions, it is easy to understand that an uncontrolled acidosis due to similar causes might be very detrimental to the animal in which it developed. In fact, Forbes (3 and 4) and others (6) (11) (13) some 20 years or more ago showed the disastrous effects of diets unbalanced with respect to mineral content, especially in the case of swine, animals which are often made to subsist largely on a grain diet.

The urine of cattle ordinarily contains sufficient bicarbonate to liberate 200 cc. or more of CO_2 in each 100 cc. of urine. If the bicarbonates of the blood from which that of the urine is derived have been used to neutralize an excess of invading acids the CO_2 content of the urine is greatly reduced, or may virtually disappear. On the other hand, if ammonia is used to neutralize the undesirable acidity, the very small amount of ammonia which is normally present in the urine is greatly increased, sometimes a hundredfold or more, as has occurred during this experiment. These processes of adjustment occur at the same time, sometimes one and sometimes the other predominating.

Although, as may be seen by reference to tables 1 and 4, some high ammonia values have been consistently obtained on exclusive grain feeding also when feeding mineral acids. The very highest ammonia values have been observed in the case of cows on otherwise normal feeding which have suddenly gone off feed (table 1, lines 15, 16, and 17). Abnormally high ammonia values are also sometimes encountered immediately following an abrupt and radical change in feeding which disappear when the animal becomes accustomed to the new ration. Most of the rations used in this experiment have been used at least two or three weeks to avoid too hasty conclusions from this cause. Blatherwick (1) found a decidedly acid response from exclusive silage feeding but expressed surprise that this should be the case in view of the somewhat alkaline character of the ash. He attributed this effect to a possible incomplete use of the silage acids. We believe that the explanation of his anomalous results lies in the fact that he made very radical changes in his rations at only very short intervals.

The pH of bovine urine seems to range normally between 7.0 and 8.0. We have observed values on the exclusive grain feeding and the feeding of the mineral acids as low as 5.6–5.8; also, values up to 8.2 or 8.3 on rations consisting mostly of legume hay.

One form of acidosis which frequently accompanies diabetes and which may be induced by abnormal diets, particularly by the eating of large amounts of fat and very little carbohydrates, causes a urine containing acetone and other bodies of similar nature derived from the incomplete oxidation of fats. These compounds may be collectively determined and designated as "Organic Acids." Normal urine gives relatively small

values for this determination which will vary somewhat with the composition of the ration. In cases of acidosis due to the incomplete use of fats in the body this value is tremendously increased.

Indications of acidosis will appear in the urine long before they could surely be detected in the blood, and the degree of variation found in the urine is much greater than would ever be tolerated in the blood. Conversely, the evidences of an acidic condition such as we show here are not to be considered as sufficient evidence that the animal is really suffering from acidosis. The conditions provoking these symptoms in the urine might be continued for some time before all the various safeguards to the normal reactions of the blood would be overcome and the gross symptoms of acidosis appear.

The absence of decided abnormality in all of these four urinary analyses, on the other hand, may usually be considered as sufficient evidence of the absence of acidosis.

THE PRESENT EXPERIMENTS

The experiments here reported were carried out as two distinct units: a summer experiment in which lactic acid, acetic acid, and three of the mineral acids were fed to cows on a pasture-grain-and-dried-beet-pulp ration, and a winter experiment on different cows which involved the use of various single feeds and combinations of these feeds.

The feeding of lactic and also of acetic acid was started at a level of $\frac{1}{8}$ pound at each feeding or $\frac{1}{4}$ pound per day. The acetic acid used was of C. P. quality and 80 per cent strength, and the lactic acid was of edible quality and 44 per cent strength when purchased. Each of these acids was diluted with water so that 1 liter of solution represented 1 pound of the pure acid. Five hundred and thirty-six cc. of the acetic acid and 944 cc. of the lactic acid were required per liter of solution.

Five pounds of dried beet pulp was substituted for a like amount of grain in the ration of the experimental cows. The carefully measured amount of acid was mixed with the water used to make the grain and dried beet pulp into a wet mash. Four liberally producing Holstein cows in early to mid-lactation which were on pasture were selected for the experiment. Two of the cows were continued on the pasture-grain-beet-pulp ration without acid to serve as control animals. One of the remaining cows received lactic acid and the other acetic acid, as just described.

The amount of each acid fed was increased by steps of $\frac{1}{4}$ pound a day after an interval of four or five days on each level, until a level of $1\frac{3}{4}$ pounds daily of each acid was reached. At this level of acid feeding the cows began to refuse small amounts of the wet mash containing the acid and it was evident that no increased amount of acid would be taken. The cows received $1\frac{1}{2}$ pounds or more of the acid a day for a period of approximately three weeks.

The acid feeding was then dropped and the two cows returned to the control ration. The other two cows which had been receiving the control ration of pasture-grain-beet-pulp were started on the acid feeding, beginning at the ¼ pound a day level. Their response to the acid feeding was very much the same as that of their mates, 1¾ pounds a day apparently being the limit of tolerance for the acid feeding.

Urine samples representing two consecutive periods of two hours each were collected from each of these cows according to the method described by Nibler and Turner (9). Samples were collected on normal feeding and on one or more occasions following each successive increase in the amount of acid fed. These samples were analyzed for pH, organic acids, ammonia, and CO_2 as bicarbonates.

The methods of analysis used in this work were as follows: The pH was determined colorimetrically with the Hellige apparatus; the organic acids by the method of Van Slyke and Palmer (18); the CO_2 as bicarbonate was determined by the use of the Van Slyke (15) blood-gas apparatus, the procedure and calculations being adapted from the method of Van Slyke and Cullen for CO_2 capacity of the blood serum (16). Ammonia was determined by an aspiration method which may be considered as a modification of either the Folin (2) or of the Van Slyke and Cullen (17) aeration procedures. Ordinarily, 25 cc. of urine were taken (in some of the urines of extremely high ammonia content as little as 0.5 cc. were used and diluted to 25 cc.) and 5 cc. of 20 per cent Na_2CO_3 solution were added; also, 2 drops of capryl alcohol as an antifoam agent. The ammonia set free was aspirated into another tube containing a measured amount of N/140 sulphuric acid. The excess of acid was titrated with N/140 ammonia, using a carefully neutralized and very dilute solution of Methyl red as indicator. A very distinct end point and highly satisfactory results are obtained by this procedure. The results of these analyses are presented in table 1, only the results obtained at the higher levels of acid feeding being shown.

Previously it has been shown by this department (10) that the feeding of as much as 50 pounds daily of corn silage in connection with alfalfa and grain over a period of several months produced no evidence of acidosis. The results here shown in table 1 represent the effects of feeding in pure form 1.5 pounds or more of either lactic or acetic acids, which are the predominant acids found in silage. The total acidity of corn silage as determined by titration averages about 2.5 per cent and rarely exceeds 3 per cent. This titration value includes the effect of a considerable amount of "buffer substances," so that the amount of true acids present is considerably less than shown by titration, and seldom exceeds 2 per cent. Since 40 to 50 pounds of silage per cow per day represents heavy silage feeding, when grain and hay are also fed, and 75 pounds per day is about as

TABLE 1

Summary of urine analyses—effects of acid feeding

LINE NO.	COW NO.	FEEDING	pH VALUE	AMMONIA NITROGEN (MG. IN 100 CC.)	CO_2 AS BICARBONATES (CC. IN 100 CC.)	ORGANIC ACIDS (CC. N. ACID IN 100 CC.)	NO. SAMPLES	DATES INCLUDED
		Control = Pasture, grain and beet pulp						1931
1	276	Control	8.05	1.36	220	22.9	8	7/6–8/10
2	343	"	7.45	2.83	140	14.4	10	9/1–10/8
3	346	"	7.40	3.59	147	23.9	6	9/1–9/16
4	368	"	8.00	2.06	167	14.4	11	7/6–8/11
5		Average	7.73	2.46	168	18.9		
		Control +		Lactic Acid				
6	276	Lactic acid	7.63	1.67	180	14.8	5	9/1–9/16
7	346	1.5–1.75 lb.	8.10	3.45	245	31.7	4	9/1–8/11
8		Average	7.86	2.56	212	23.2		
		Control +		Acetic Acid				
9	343	Acetic acid	8.0	2.82	173	18.2	4	8/1–8/11
10	368	1.5–1.75 lb.	7.2	3.65	69	16.7	5	9/1–9/15
11		Average	7.6	3.23	121	17.4		
		Normal Ration +		Mineral Acids				
12	368	2–3 oz. HCl	6.8 –	20.0	5	13.0	2	9/25
13	346	2–3 oz. H_3PO_4	6.9	7.4	30	19.9	2	9/22 and 9/25
14	276	3 oz. H_2SO_4	6.9	7.9	15	18.0	2	9/25
		Normal Ration +		Cows off Feed				
15	276	Lactic Acid	7.8	125.0	98		1	9/11
16	343	Grain and Silage/$NaHCO_3$	7.9	27.0	49		1	3/22
17	236	Grain and Silage	6.8 –	80.0 +	22	14.8	1	10/20/1931

much as will be eaten regularly by a large cow on exclusive silage feeding, therefore, the amount of acid fed in this experiment was as much as a cow would be likely to obtain from eating corn silage. As will be seen in table 1, lines 6, 7, and 8, the bicarbonates increased slightly and the ammonia changed very little on the lactic acid feeding in comparison with the results from the same cows on the same ration without the acid. The greater observed change is in the opposite direction to one likely to be caused by acidosis. Acetic acid feeding as shown in lines 9, 10, and 11 caused some decrease in bicarbonates and some increase in ammonia, in comparison with the normal ration, but the amount of variation was not sufficient in either case to constitute an abnormal condition. In short, there is no evidence of acidosis in either case. The pH and organic acids were also normal, indicating that both of these acids are metabolized and used without difficulty by the dairy cow.

When the results for the normal feeding shown in lines 1, 2, 3, and 4 are properly arranged for this purpose, there can be readily detected a minor seasonal influence on the composition of the urine which was probably due to the change in the condition of the pasture. The amount of the variation, however, is not sufficient to affect the interpretation of the acid-feeding experiment. At the conclusion of this work with lactic and acetic acids one of these cows was fed first two ounces daily, then 3 ounces daily of hydrochloric acid; another was fed like amounts of sulphuric acid, and a third was fed like amounts of phosphoric acid. These mineral acids were also fed by mixing them with the water used to wet the mash of grain and beet pulp. It was very evident that only small amounts of the mineral acids would be taken and since the effect of even these small quantities on the composition of the urine was very marked the feeding was soon discontinued. Hart, Steenbock, Kline and Humphrey (6), Sommer and Hart (12), and Lamb and Evvard (8) also report the feeding of hydrochloric or sulphuric acid to cattle and swine, in amounts comparable to those used in this experiment in some cases for an extended period without serious effect, though death was produced in rabbits from too heavy acid feeding.

When only about 1/10 as much was fed as in case of the lactic or acetic acids in this experiment, the bicarbonates were greatly reduced and the ammonia content decidedly increased. The result was most marked in the case of hydrochloric acid. These results are also shown in table 1. In addition to the results as there shown, it may be stated that determinations of chlorides, sulphates, and phosphates were made by standard methods of analysis on the urine of the cows when receiving a normal ration and again when receiving the acid. In each case the mineral-acid feeding resulted in a marked increase of the corresponding ingredient of the urine.

In the other section of the experiment five cows more advanced in lactation were used. Among the single feeds and combinations studied were silage and grain, silage alone, grain alone, timothy hay and grain, timothy hay and molasses, alfalfa hay and corn, soybean hay and corn, and clover hay and corn. Several days of adjustment to the ration were allowed in each case before the sample of urine intended to represent the effect of the ration on the urinary composition was taken. Each type of feeding was used, as a rule, on at least two cows and in most cases several samples of urine were collected and analyzed from each cow on each type of feeding. The methods of collection and analysis already described were also used in this portion of the work.

Such radical changes in ration as were used in this group of experiments are, to say the least, not conducive to liberal or long continued production. Although an effort was made to make the various changes with as little disturbance as possible, the decline in milk production was very striking. The results of the urinary analyses are shown in tables 2, 3, and 4.

To serve as normals for purposes of comparison the average analysis of samples from 20 different cows on an alfalfa-grain-silage ration was selected. These values are shown in line 1 of table 2.

The next values shown, lines 2 to 10, table 2, for silage and grain feeding show an average reduction from the normal value of more than 100 cc. of CO_2 and a considerable increase in ammonia. The results in both cases, however, still come well within the range of what may be considered as normal values for these constituents.

Next following, lines 11 to 14, table 2, will be found a set of results from cow 236 on the same ration at a different time. The results are seen to be strikingly different from the others.

The results in lines 2 to 10 were obtained as the result of 18 days' feeding of a silage-grain ration, following normal feeding. No abnormal condition was produced in that length of time. The results from the same ration in lines 11 to 14 were obtained following a period of exclusive grain feeding, during which the bicarbonates had been reduced to a very low level and the ammonia raised to several times its normal value, as shown in lines 1, 2, and 3, table 4. Although in the one case the silage seemed to prevent or at least greatly delay the development of acidic symptoms, it was in the other case able to correct the acidic symptoms developed on the exclusive grain feeding, only rather slowly. The experiment as described for cow 236 was also carried out with another cow, with substantial duplication of the results shown for No. 236.

The values substantially above 100 cc. of CO_2 for exclusive silage feeding, as seen in lines 15–17, table 2, may be considered as low normal values.

TABLE 2

Summary of urine analysis—effects of different feeds

LINE NO.	COW NO.	FEEDING	pH VALUE	AMMONIA NITROGEN (MG. IN 100 CC.)	CO_2 AS BICARBONATES (CC. IN 100 CC.)	ORGANIC ACIDS (CC. N. ACID IN 100 CC.)	NO. SAMPLES	DATES INCLUDED
				Normal Feeding				
1	Av. 20	Alfalfa Silage, grain		1.8	267	29.1	20	1/10–15 1930
				Silage and Grain following Normal Feeding				
2	236	Silage + grain	7.35	1.03*	111	19.2	5	10/15–11/2
3	323	" "	7.45	2.26	172	36.2	3	10/15–10/26
4	351	" "	7.35	3.84	190	35.1	5	10/15–11/2
5	314	" "	7.30	1.20	135	9.7	2	11/10–11/12
6	356	" "	7.36	1.42	226	32.8	5	10/15–11/2
7	366	" "	7.20	2.62	126	13.2	2	11/10–11/12
8	343	" "	7.22	4.34	110	23.5	5	10/15–11/2
9	397	" "	7.30	3.67	118	20.5	4	10/20–11/2
10		Average	7.31	2.55	148	23.7		
				Silage and Grain following Exclusive Grain feeding				
11	236	Silage + grain	5.8	39.5	3		1	1/5/32
12	236	" "	5.6	45.6	4	13.4	1	1/7/32
13	236	" "	7.0	8.0	11		1	1/14/32
14	236	" "	7.2	12.7	50		1	1/22/32
					Silage only			
15	314	Corn Silage	7.54	1.34	142	12.9	2	11/15–12/7
16	366	" "	7.60	0.68	119	9.2	2	11/15–12/7
17		Average	7.57	1.01	131	11.1		

* One abnormal value was not included in this average.

Taken in connection with the low ammonia value for this ration, and normal values for pH and organic acids, it seems safe to conclude that this ration would never develop a case of acidosis. With timothy hay added to this ration, as shown in lines 1, 2, and 3, table 3, neither the ammonia nor the CO_2 values are greatly changed. The likelihood of acidosis would, therefore, seem about the same as for the exclusive silage feeding.

Replacing the timothy by alfalfa, lines 4–7, table 3, increased the bicarbonates to the high average level of 368, the ammonia being correspondingly reduced to a very low level.

Replacing the silage by grain in the next ration, lines 8–11, table 3, lowered the bicarbonate level about 150 points and nearly tripled the ammonia content of the urine.

In the next ration of timothy hay and grain, lines 12–15, table 3, it will be noted that molasses is also included. It was added primarily to increase the palatability and consumption of the timothy hay. Only a small amount was used and we have not been able to ascribe any specific effect to its use. Again, this replacing of alfalfa hay by timothy hay lowered the bicarbonate level nearly 150 points, and nearly doubled the ammonia value, bringing it to 5 times its value on the silage only, or silage and alfalfa hay ration.

On timothy hay plus only the small amount of molasses added to improve its palatability, lines 16 and 17, table 3, the CO_2 was increased to a full normal level and the ammonia decreased to a very low level, showing that timothy hay alone is alkaline rather than acidic in its effect. Its behavior in the timothy and grain ration, however, shows that it does not have a very pronounced effect in neutralizing the acidic effect of other feeds.

On the next ration shown, grain only, lines 1–3, table 4, the bicarbonates reached a very low level and the ammonia an unprecedented high level. The pH on this ration was very low, about 5.6; in fact that ration had most of the earmarks of an acidosis-producing ration, judged by the composition of the urine. After feeding this ration for about 3 weeks, samples of blood from these cows were run for CO_2 capacity. The values found indicate, according to Van Slyke's interpretation of like data in humans (9), a mild cnodition of acidosis unaccompanied by severe symptoms. At this point the ration was discontinued and our data do not answer the question whether severe acidosis would have developed on the continued use of this ration. The distortion of the data for ammonia and bicarbonates on the exclusive grain feeding was less marked in the case of cow 397 than in case of the other cows. Although she was fed only grain like the others it developed that through an oversight she had been kept in a straw-bedded stall. The other cows and those standing near them were bedded with shavings.

TABLE 3

Summary of urine analysis—effects of different rations

LINE NO.	COW NO.	FEEDING	pH VALUE	AMMONIA NITROGEN (MG. IN 100 CC.)	CO_2 AS BICARBONATES (CC. IN 100 CC.)	ORGANIC ACIDS (CC. N. ACID IN 100 CC.)	NO. SAMPLES	DATES INCLUDED
				Silage and Timothy Hay				
1	314	Silage + Timothy	7.56	2.09	84	8.6	6	12/10–12/28
2	366	" "	7.96	3.33	153	11.8	6	12/10–12/28
3		Average	7.76	2.71	118	10.2		
				Silage and Alfalfa				
4	236	Silage + Alfalfa	7.70	0.76	414	28.7	2	11/3–11/12
5	343	" "	7.65	0.34	257	16.8	2	11/3–11/12
6	397	" "	7.70	1.78	432	17.5	2	11/3–11/12
7		Average	7.69	0.96	368	21.0		
				Alfalfa and Grain				
8	236	Alfalfa and Grain	7.66	3.46	195	18.5	3	11/15–11/24
9	343	" " "	7.70	1.19	240	15.7	3	11/15–11/24
10	397	" " "	7.66	3.53	208	15.6	3	11/15–11/24
11		Average	7.68	2.73	214	12.5		
				Timothy and Grain/Molasses*				
12	314	Timothy, Grain + Molasses	7.7	2.00	127	17.6	4	1/9–2/4
13	366	" " "	7.5	4.29	101	14.0	4	1/9–2/4
14	397	" " "	6.8	8.20	3	6.6	3	12/22–1/7
15		Average	7.3	4.83	77	12.7		
				Timothy/Molasses*				
16	314	Timothy + Molasses	7.95	0.20	161	6.6	2	1/2–1/7
17	366	" "	8.10	0.28	277	10.4	2	1/2–1/7

* In both of these rations the molasses was fed only in small amounts to improve the palatability of the timothy hay.

In the following group of data, lines 4–8, table 4, from cows continuously receiving rations consisting only of corn and one or the other of the legume hays, the alkaline character of the legume hays is shown both by high bicarbonates and low ammonia values in the urine produced. This is true of all the rations containing legume hay. Another notable feature is the relatively higher content of organic acids in the urine whenever legume hay was being fed. No explanation of this finding is known to us, but the observed level of organic acids is not high enough to constitute an abnormal condition.

High protein rations have been popularly assumed by many to bring about an acidic condition. Certainly, however, any ration high in protein because of legume hay will be quite the opposite of acidic. In one phase of this experiment not shown in detail the protein content of the grain mixture supplied to two cows was doubled from about 14 per cent to about 28 per cent protein content. Even when this high protein grain was freely fed in rations of high grain content, no increase of acidic symptoms was noted beyond those produced by like feeding of the grain mixture of lower protein content.

In lines 9 to 14, table 4, are shown some of the results obtained by the addition of mineral supplements to some of the above rations with a view to correcting the acidic tendencies displayed. The degree of success achieved may be estimated by comparison with the corresponding ration without the mineral supplement as shown elsewhere in the tables, and the normal value as shown in table 2, line 1. In a few instances, for example cow 236, just following the results shown in lines 11 to 14, table 2, the addition of 2 ounces of bone meal quickly brought the CO_2 and ammonia values of the urine to practically a normal level. The continued use of either bone meal or hydrate lime or sodium bicarbonates, however, failed to give results which seemed altogether satisfactory in restoring the constituents of the urine to their normal values. When these materials were fed in connection with an acidic ration in the amounts indicated, the ammonia values were double or triple the normal value and the CO_2 per 100 cc. of urine was only a small fraction of the usual amount.

CONCLUSIONS

The tentative conclusions to be derived from this series of experiments are, in our judgment, as follows, though it is recognized that in some respects the data are not sufficient in amount to justify final conclusions:

None of the characteristic symptoms of acidosis were produced in the urine by the feeding of 1.5 to 1.75 pounds daily of either lactic or acetic acids in connection with an otherwise normal ration. This is more acid than a cow would be likely to ingest from eating silage.

TABLE 4

Summary of urine analysis—effects of different rations

LINE NO.	COW NO.	FEEDING	pH VALUE	AMMONIA NITROGEN (MG. IN 100 CC.)	CO_2 AS BICARBONATES (CC. IN 100 CC.)	ORGANIC ACIDS (CC. N. ACID IN 100 CC.)	NO. SAMPLES	DATES INCLUDED
				Exclusive Grain Feeding				
1	236	Grain only	5.8	46.1	17	12.6	5	12/12–12/28
2	343	" "	5.8	28.8	11	9.7	5	" "
3	397	" " *	7.6*	4.13*	67	7.4	4	12/12–12/21
				Corn and Legume Hays				
4	318	Alfalfa + Corn	8.0	1.24	348	20.6	2	continuous
5	334	S. B. Hay + corn	8.05	0.60	484	29.0	2	"
6	341	Clover hay + corn	8.05	0.44	459	31.2	2	"
7	342	" " "	8.0	0.48	521	40.0	2	"
8	350	" " "	7.95	1.02	335	32.6	2	"
		Timothy and grain		Mineral Supplements				
9	397	+ 2 oz. bone meal	6.9	5.81	10	11.2	4	1/13–2/4
10	397	+ 2 oz. $Ca(OH)_2$	7.6	4.15	46		2	2/8–2/26
11	397	+ 2–6 oz. $NaHCO_3$	7.8	4.70	79	16.8	4	2/27–4/4
		Silage, grain						
12	236	+ bonemeal, 2 g	7.6	3.40	94	16.0	2	1/23–2/4
13	236	+ 2 oz. $Ca(OH)_2$	7.7	3.40	70		1	2/7–2/16
14	236	+ 2–6 oz. $NaHCO_3$	7.7	3.54	85	18.0	5	2/19–4/4

* Cow 397 was fed only grain but through an oversight was kept where she obtained some straw intended for bedding.

The feeding of either of the three mineral acids, hydrochloric, sulphuric, or phosphoric, to the extent of only two or three ounces daily soon developed pronounced symptoms of acidosis and marked increase of the corresponding acid radical in the urine.

Silage fed alone or in combination with other feeds does not produce in the urine any of the charactieristic symptoms of acidosis. When fed with a ration which by itself soon produced the acidic symptoms silage prevented or at least greatly delayed the appearance of such symptoms. After pronounced signs of acidosis had been produced by another ration the addition of corn silage gradually reduced the urinary analysis to a condition approaching normality.

All the leguminous hays studied seemed to exert a decidedly alkaline effect on the urine, while timothy hay seemed to be in about the same class with silage in this respect.

The feeding of an exclusive grain ration quickly developed in the urine marked symptoms of acidosis as indicated by the practical disappearance of bicarbonates and the increase of the ammonia to twenty or more times the normal level.

Grain fed in connection with any roughage studied seemed to depress the bicarbonates, and increase the ammonia content of the urine.

The protein content of the grain did not seem of particular influence in this respect.

Alkaline mineral supplements, so far as they were tried, seemed relatively unsatisfactory in correcting the results of an acidic ration.

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THE EFFECTS OF SOME FLY SPRAYS ON THE HIDES AND ON THE BODY TEMPERATURES OF DAIRY COWS[1]

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Many farmers are using fly repellent oils during the summer months to protect their dairy cattle from flies. To be valuable for this purpose an oil must:

1. Effectively repel flies.
2. Cause no physiological disturbance which will depress milk production.

The first factor is, of course, fundamental to the use of fly sprays. The necessity of considering also the physiological influence of the oil is pointed out by Freeborn *et al.* (2), who found that certain fly sprays caused a greater decrease in milk production than the flies did.

An experiment to investigate the effect of several prospective fly sprays in regard to these two factors was carried on at Iowa State College during the summer of 1931. This paper deals only with the physiological effect of the oils. The determination of the fly repellent value of these sprays is reported in another paper by Pearson, Wilson, and Richardson (5).

The methods used in this experiment to measure the physiological influences of the oils consisted of:

1. A determination of the effect of the oil upon the skin and hair of the sprayed animals.
2. An investigation of the results of the sprays upon the body temperatures of the animals as measured by rectal temperatures.

No doubt milk production would be the ideal measure of the physiological effect of fly sprays. However, the many and varied factors influencing milk production make necessary detailed and elaborate control of experimental conditions in order to secure accurate results. It was believed that the two methods used would determine the more important physiological effects of the fly sprays without being influenced by other factors which alter milk production. In addition, a much more extensive study was possible by disregarding the period of lactation of the animals.

SKIN INJURY STUDIES

Twelve petroleum oils varying in viscosity and degree of refinement and containing various added chemical repellents were used in the trial. These were sprayed on the milking animals of six farm herds in the vicinity of

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Ames, Iowa. Most of the cattle were Holsteins, although one herd of Guernseys was included. The cows were divided into twelve groups of seven to eight cows each, as uniform as possible in regard to color, breed and size of animal. Each group contained cows in at least two different herds. One oil was allotted to each group and sprayed continuously on that group for two months. Any extra cows which were not included in the twelve groups were used as unsprayed check animals or were sprayed with oil No. 1, which served as a check oil in every herd. The cows were kept in the barn only at milking time or during stormy weather. The remainder of the time they were allowed to run in pastures.

The sprays were applied once a day at the rate of two fluid ounces (approximately 60 cc.) per cow. Where electricity was available small electric sprayers were used. These delivered the required amount in approximately one minute, although the time varied somewhat with the temperature and viscosity of the oil. Where electricity was not available, hand pressure sprayers which delivered a continuous spray were used. Three herds were sprayed in the morning and the other three in the afternoon. The groups were so arranged that each oil was sprayed with both types of machines and at both times of the day. An effort was made to distribute the oil as uniformly as possible over the entire body of the cow. The cows were inspected daily for any indication of skin injury due to the fly spray.

The first appearance of skin injury occurred some 35 days after the beginning of the spraying period. The visible evidence consisted of an unusually large amount of loose hair on some animals. Several individuals showed bare spots where the hair had entirely fallen out. These spots were irregular and relatively small, ranging up to about one inch in diameter. They were restricted almost entirely to the regions of the back, loin, hooks, and rump. Although the bare spots were not very numerous, the coats of most of the animals so affected showed an unusually large amount of loose hair in these regions.

Another type of injury consisted of a reddening and roughening of the skin. The skin showed an unusually large amount of scurf and tended to crack and peel in places, as if sunburned. The appearance of this trouble was most marked on areas of white skin were evidence of reddening could be better detected. The cracking and peeling also occurred on the darker colored areas though seemingly it was less severe.

An effort was made to determine if there was any thickening of the skin during the time the injury was appearing. Caliper measurements taken at the end of the first and second months of spraying showed no consistent changes. The earlier effects of the sprays on the skin thickness were not determined.

Only five of the twelve oils gave visible evidences of causing skin injury. Two of these caused only a relatively small amount of irritation, as one

caused a slight skin burning on 2 out of 29 cows sprayed, while the other burned to a noticeable degree the skin of 1 out of the 8 cows sprayed with it. The other three sprays produced relatively severe burning on several cows. The use of the remaining seven oils resulted in no injury to the skin. The hair of nearly all the sprayed cows appeared somewhat harsh and dirty due to the accumulation of dust on the oily hair. This was washed off with each rain and probably caused little or no harm. The number of cows injured by each spray and the relative degree of injury are shown in table 1. This table also shows the increases in body temperature caused by spraying with each oil.

TEMPERATURE STUDIES

The effects of the various fly sprays upon the body temperature of the cows were determined by spraying in the morning and recording the rectal temperature of the animals at hourly intervals thereafter. The daily temperature curves for thirteen unsprayed cows were determined. These same cows were used later for measuring the effect of spraying on body temperature. Eleven of these cows were then sprayed at 6 A. M., each with a different oil, while two were left unsprayed as checks. The body temperatures of these cows were then taken at hourly intervals for 10 hours. The spraying and body temperature readings were repeated three more

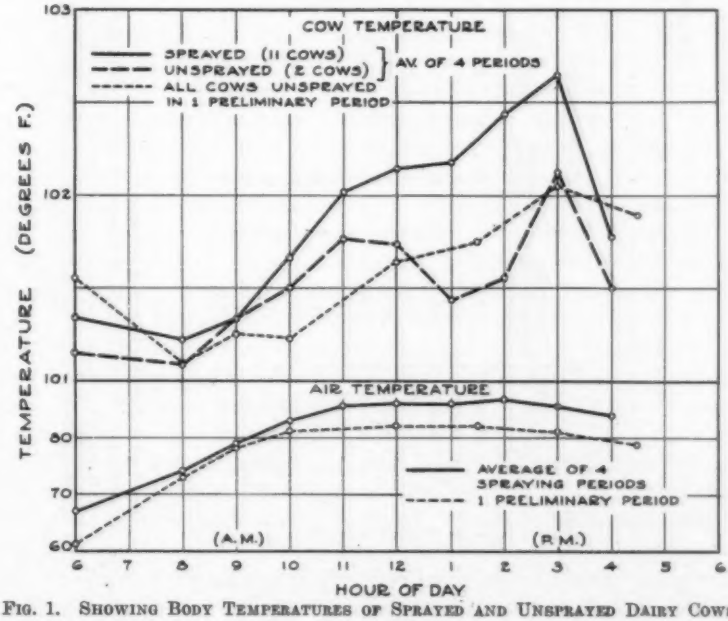

FIG. 1. SHOWING BODY TEMPERATURES OF SPRAYED AND UNSPRAYED DAIRY COWS.

TABLE 1

Showing the effect of fly sprays on the skins and on the body temperatures of dairy cows

SPRAY NO.	COMPOSITION OF SPRAY				SKIN INJURY*			INCREASE OF BODY TEMP. (DEGREES F.)†	
	Oil		Added Chemical		No. Cows Showing Injury				
	Viscosity (Saybolt at (100° F.)	Unsulphonatable Residue (Approx.)	Repellent Ingredient	Toxic Ingredient‡	Marked	Slight	None	Average Increase During 10 Hours After Spraying	Increase at Point of Highest Temperature
1	80–85	70%	A	2X		2	27	.32	.53
2	40–45	80%	A	2X			7	.22	.73
3	80–85	70%	B	X			8	.30	.80
4	50–55	70%	A	2X			8	.75	1.13
5	80–85	90%	A	2X	1		7	.18	.63
6	80–85	70%	C	X	3		5	.91	2.00
7	80–85	70%	D				7	.46	1.50
8	80–85	70%	E	X			8	.41	1.00
9	80–85	70%	F	X			7	.36	1.25
10	80–85	70%	G	X	1	1	6	.12	.48
11	80–85	70%	H	2X			7	.46	1.23
12	80–85	70%	I	X	2	2	4	—	—
Unsprayed Check							11	−.19	.66

* Based on a 2-month spraying period.

† Comparison of temperature of 4 sprayed cows against the temperatures of the same cows unsprayed.

‡ X Refers to a given concentration of the toxic ingredient.
2X Refers to twice the concentration of the same ingredient.

times, but the sprays were rotated so that each spray was used on four different cows. Between the trials the cows were washed thoroughly with soap and water to eliminate any effect of the oils used previously.

Air temperatures were recorded at the same time that the temperatures of the animals were taken. During the trials the cows were allowed to run in a small pasture with no shade available. Since the cows were very gentle, little or no exictement was caused by the handling necessary to obtain their temperatures. The increased temperature of the cows due to the effects of a given spray was determined by comparing the hourly temperatures of the unsprayed cows with the temperatures of the same cows sprayed.

A small but consistent rise in the body temperatures of the sprayed cows occurred after spraying as is seen in table 1. The increase was not most pronounced at the warmest part of the day. The viscosity of the oils seemed to have no significant effect upon the variation of temperature resulting from spraying, although the more highly refined oils were included among those inducing the least temperature rise. Figure 1 shows graphically the average temperature of the sprayed and unsprayed cows as well as the temperature of the entire group during the preliminary period.

DISCUSSION OF RESULTS

In this experiment it was noted that visible evidence of skin injury did not appear until the second month of spraying. It would seem that the injurious results of fly sprays are not manifested immediately, but occur as a result of constant spraying. Perhaps repeated application of the oils causes the accumulation of a spray residue on the skin. As this residue increases, the harmful effects of the spray begin to develop. Two oils (nos. 2 and 4) used in the experiment were of lighter viscosities (45 and 55 seconds) than the rest of the oils (85 seconds). Neither of these two oils showed any evidence of causing skin injury. Probably the lighter oils left a smaller amount of residue upon the skin. However, among the oils of higher viscosity, injury seemed to be connected with the added chemical ingredients. With oils of the same viscosity and chemical ingredients (nos. 1 and 5) the more highly refined oil showed a slightly greater amount of injury. Evidently in this case the additional refinement did not remove the substances causing the injury.

The type of sprayer used in applying the oil had a noticeable influence on the injurious qualities of the sprays. The harmful results were confined to the cows sprayed with electric sprayers. These sprayers delivered the spray with considerable force tending to cause the oil to penetrate to the skin. In contrast, the hand pressure sprayers delivered a misty spray which tended to cling to the outside edges of the hair rather than penetrating

to the skin. Freeborn *et al.* (1) have suggested that oil coming in direct contact with the skin is more likely to cause injury.

The region of the animal on which the most severe skin injury occurred would lead one to believe that the rays of the sun are very closely related to the injury caused by sprays. The injury was confined almost entirely to the backs, loins, hips and rumps of the animals, the points upon which direct sunlight falls. There was probably also a greater tendency for the oil to reach the skin in these regions since they are nearly level and consequently the oil would not tend to run off as it would on the lower portions of the body. As care was taken to always apply the spray as uniformly as possible it is believed that these results were due to the effect of the sun and to greater penetration of the oil, rather than to uneven application of the spray.

Evidently oils applied to the skin of a cow impede the loss of body heat by this channel. Freeborn *et al.* (3) have found that the body temperatures of sprayed cows were more closely correlated to the air temperature than were those of unsprayed cows. In a later paper (1) these authors have suggested that the oil has a physiological effect on the skin which impairs its ability to aid in maintaining body temperature. Melvin (4), however, attributes the increase in body temperature of sprayed cattle to the effect of direct sunlight as well as to that of the actual air temperature. His data show that a given air temperature caused much greater elevation of body temperature of sprayed cattle when the animals were exposed to the sun than when they were in the shade. He also found that when cattle were exposed to the sun after spraying the rise in temperature of dark colored animals was greater than that of lighter colored ones.

It would seem logical to suppose that the oil prevents the radiation of heat by forming a coating over the surface of the skin. Perhaps, also, the oil soaked hair prevents the circulation of air next to the skin, thus acting as an insulating medium. Additional absorption of light rays when the cow is exposed to the sun would tend to raise the temperature of the body, which, with the cooling mechanism impaired, would cause an additional rise in body temperature.

The elevations in temperature occurring in the trial reported here were not sufficient to cause dangerous results. It must be admitted, however, that extremely high air temperatures were not encountered. The average daily maximum air temperature in this trial was 86° F., although on one day the thermometer reached 92°. These temperatures are sufficiently high to give significant results, but do not give extreme results such as Melvin (4) reports at higher temperatures.

SUMMARY

Twelve fly repellent oils sprayed at the rate of two fluid ounces per day differed widely in their physiological effects upon the skin of dairy cattle.

Seven of them caused no skin injury when sprayed over a two months' period. Five oils caused noticeable injury as indicated by loss of hair and cracking or peeling of the skin. The injury was most evident on those portions of the body most exposed to the sun's rays. The amount of injury caused by the sprays was influenced by the method of application. Cows varied in their susceptibility to skin injury.

During periods in which the maximum daily air temperatures averaged 86° F. the body temperatures of sprayed animals were slightly higher than those of unsprayed animals. The temperatures of the cows sprayed with 5 of the 11 oils did not show marked variations from those of the unsprayed cows. Application of the other 6 oils caused definite, though not dangerous, temperature rises. The elevations of body temperatures were more pronounced at higher air temperatures.

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THE EFFECT OF PASTEURIZING ON THE BACTERIAL FLORA OF THE ICE CREAM MIX[1]

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INTRODUCTION

The practise of pasteurizing market milk at 142°–145° F. for 30 minutes has been carried over into the ice cream industry because it has proved efficient in destroying organisms in milk. In recent years it has been demonstrated experimentally as well as under practical conditions that mixes were not seriously injured by the use of higher temperatures for shorter holding periods. As a result there are in use to-day, pasteurization temperatures ranging from 145° F. to 180° F. for time intervals varying from 1 to 60 minutes.

There are but few data available showing the relative efficiencies of various temperatures and time intervals for the destruction of bacteria in ice cream mixes. It is a common concept, however, that as the temperature is increased the length of time necessary to attain a given efficiency is decreased. The extent to which the period of heating may be decreased as the temperature is increased has not been definitely established. The advent of continuous and flash pasteurization has rendered this a question of vital importance.

REVIEW OF LITERATURE

In a discussion of pasteurization temperatures for ice cream mixes there are three points of major importance to consider. First, the effect of the temperature employed on the flavor, whipping quality, freezing time and overrun of the mix; second, the bacterial destruction at the temperature employed; and third, the cost and time consumed at the temperature used.

The effect of various temperatures and holding periods on the physical and chemical properties of the ice cream mix has been studied by several investigators, including Dahle and Barnhart (4), Dahle, Keith and McCullough (5), Martin, Swope and Knapp (10), Olson (14) and Hening (9). These investigators have demonstrated that there are certain benefits to be derived by employing pasteurizing and homogenizing temperatures above 145° F. Since there has been a tendency among ice cream manufacturers

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to use higher processing temperatures and to shorten the holding period, the question immediately arises as to what effect this practise has on bacterial destruction.

One of the first attempts to answer this question was made by Martin, Swope and Knapp (10), who found that high temperatures and holding periods of varying lengths (160° F. for 15 minutes to 2 hours) gave the greatest bacterial destruction, although 145° F. for 20 minutes reduced the counts materially.

Hammer and Sanders (8) determined that 145° F. for 30 minutes destroyed 91.5 to 99.5 per cent of the organisms that were present in the ice cream mix. Other investigators, including Fabian and Cromely (6) and Fay and Olson (7) (13) contended that it was necessary to pasteurize at 150° F. for 30 minutes in order to produce consistently a finished ice cream of less than 100,000 bacteria per cc. The destruction of pathogenic bacteria in ice cream as well as the reduction in total count is of major importance. This question has received the attention of many investigators in connection with milk but only a limited amount of work on the problem has been done with ice cream mixes.

The work of Park (15) who confirmed the findings of Newman, Roseman and Davis (cited by Park) together with the experiments of Meanwell (11) proved rather conclusively that 140° F. for 20 minutes destroyed the pathogenic organisms commonly found in milk.

Oldenbusch, Frobisher and Shrader (12) working with *B. diphtheriae, B. typhosus, M. tuberculosis* and hemolytic streptococci in ice cream mixes concluded that 143.5° F. for 30 minutes was sufficiently high to destroy these pathogenic bacteria and to allow an ample margin of safety.

It is evident from the results of previous investigators that the minimum temperatures employed (145° F.–150° F. for 30 minutes) are sufficient to destroy pathogenic bacteria and produce ice cream with a satisfactory bacterial count.

PLAN OF EXPERIMENT

In this experiment a standard ice cream mix containing 12 per cent butter fat was subjected to various temperatures and holding periods. Total and differential bacterial counts were made before and after pasteurization in an attempt to determine which types were destroyed. The characteristics of the organisms surviving pasteurization were also studied.

Pasteurization of the mixes. The following pasteurization exposures were used:

145° F. for 20, 25 and 30 minutes.
150° F. " 20, 25 and 30 "
160° F. " 10, 15 and 20 "
170° F. " 2, 5 and 10 "
180° F. flash.

The raw mix was first thoroughly agitated and plate counts were made using dilutions ranging from 1–1,000 to 1–1,000,000. By means of a sterile pipette, 10 cc. portions of the mix were transferred to a series of large test-tubes. Four of these tubes were prepared for each temperature, one for each of the three time intervals and one for the thermometer. By means of a small water bath which could be quickly heated, the four tubes were brought to the desired temperature, as indicated by the thermometer in the check tube. When the mix had reached the desired temperature, all four tubes were transferred to a constant temperature bath regulated to the desired temperature, $\pm 1°$ F. At the end of each holding period a tube was removed from the bath and plates were made immediately.

The same procedure was carried out for all the exposures except 180° F. In this case the mix was heated by immersing small tubes of mix in boiling water until 180° F. was reached. The sample was removed and dilutions prepared immediately.

Method of plating. The methods for volumetric analysis described by the American Dairy Science Association Committee on Ice Cream (3) were followed throughout these trials. Bacto-Nutritive Caseinate agar to which brom-cresol purple indicator (1:33333) had been added was used for the differential counts. Parallel determinations were made with beef extract agar. Duplicate plates of each medium were made from all dilutions.

It was found by preliminary experiments that 80° F. was the most suitable temperature for the differentiation of the types of bacteria. The retardation of growth of alkali formers at 80° F. lessened the tendency for the diffusion of alkali throughout the medium and hence facilitated the identification of acid formers. Incubation at this temperature reduced the size of the colonies formed by proteolytic types although they were readily distinguishable.

Caseinate agar plates were first counted after 48 hours' incubation to record the rapid acid producers and the peptonizers. At the end of five to seven days the slow acid formers and the alkali formers and inert groups were counted on the next higher dilution plate. Usually the first count was made on the 1–100 dilution plates and the final count on the 1–1,000 plates.

Some mixes were made from milk products which had been previously pasteurized and others from raw ingredients.

RESULTS OF PASTEURIZING MIXES MADE FROM INGREDIENTS WHICH HAD BEEN PREVIOUSLY PASTEURIZED

The figures given in table 1 are an average of three trials with a mix of standard composition which had been made of pasteurized skimmilk and cream, sweetened condensed skimmilk, sugar, and gelatine. It will be seen by observing the counts that the percentage of destruction was rather low,

ranging from 74.3 to 86.0 per cent. This is to be expected since the mix was made from pasteurized ingredients and most of the organisms present had survived the previous pasteurization.

TABLE 1

Effect of various pasteurization exposures on the total and differential count of an ice cream mix (average of three trials)

Raw mix count, 23,000 on standard agar; 208,250 on caseinate agar

PASTEURIZATION		STANDARD AGAR	CASEINATE AGAR PLATES				
				Per cent distribution of types surviving			
Temp. ° F.	Time	Per cent destroyed	Per cent destroyed	Rapid acid	Slow acid	Peptonizer	Alkali and inert
145	20	82.73	74.30	....	55.14	2.99	41.87
145	25	84.13	78.39	....	33.33	3.44	63.23
145	30	88.10	81.75	....		4.60	
150	20	86.73	85.11	....	54.83	8.70	36.47
150	25	88.04	86.07	....	55.17	5.86	38.97
150	30	87.08	85.11	....	58.06	5.96	25.98
160	10	83.79	81.27	....	70.08	4.23	25.69
160	15	89.49	83.79	....	64.19	5.34	30.47
160	20	89.91	88.47	....	60.41	4.79	34.80
170	2	88.47	83.19	....	32.85	5.14	62.01
170	5	89.85	83.55	....	43.79	3.94	52.27
170	10	89.85	85.59	....	54.44	4.50	40.16
180		86.23					

Further, it will be observed that the temperatures and time intervals employed did not yield results sufficiently different to be measured by the plate count.

Pasteurization destroyed all of the rapid acid producers, whereas large numbers of slow acid formers survived. Many of the peptonizing, and alkali forming and inert groups withstood pasteurization, the number surviving being rather constant at each exposure employed.

It will be seen that the slow acid formers constitute the largest percentage of the organisms surviving pasteurization. In only three instances did this group constitute less than 50 per cent and in eight instances more than 50 per cent of the flora after pasteurization. The peptonizing group constituted but a minor portion of the flora, whereas the alkali forming and inert group made up from 25.7 to 63.2 per cent of the flora.

A comparison of the caseinate and extract agar counts reveals that the former are approximately nine times as high as the latter.

The results presented in this table are similar to those obtained with three other mixes made from ingredients which had been previously pas-

teurized. With two of these the initial counts as well as the percentage destruction were higher than the one presented in table 1. In the other mix the initial count was lower and also the percentage destruction was lower. In all instances the rapid acid producers were completely destroyed, and the flora after pasteurization consisted largely of slow acid formers.

RESULTS OF PASTEURIZING MIXES MADE FROM RAW DAIRY PRODUCTS

The data shown in table 2 are an average of seven trials with uniform mixes made of raw ingredients. It will be seen that the bacterial counts on the raw mix are extremely high and consist mostly of the rapid acid producing group.

TABLE 2

Effect of various pasteurization exposures on the total and differential count of an ice cream mix (average of seven trials)

Raw mix count, 999,142 on standard agar; 3,516,428 on caseinate agar

PASTEURIZATION		STANDARD AGAR	CASEINATE AGAR PLATES				
				Per cent distribution of types surviving			
Temp. ° F.	Time	Per cent destroyed	Per cent destroyed	Rapid acid	Slow acid	Peptonizer	Alkali and inert
145	20	99.56	99.84	.....	.....	23.59	76.41
145	25	99.60	99.87	.....	.....	35.85	64.15
145	30	99.60	99.88	.....	.....	37.66	62.34
150	20	99.55	99.87	.....	.....	34.84	65.16
150	25	99.64	99.88	.....	.....	40.45	59.55
150	30	99.58	99.89	.....	.....	39.91	60.09
160	10	99.63	99.91	.....	.....	41.15	58.85
160	15	99.65	99.90	.....	.....	44.90	55.10
160	20	99.66	99.90	.....	.....	50.40	49.60
170	2	99.62	99.90	.....	.....	47.53	52.47
170	5	99.68	99.91	.....	.....	49.11	50.89
170	10	99.68	99.92	.....	.....	51.10	48.90
180		97.51	98.46	80.30	.....	3.23	16.46
180	1	99.48	98.85	.....	.....	29.79	70.21

At all pasteurization temperatures and time intervals employed, it will be noticed that there was a marked decrease in bacterial counts. The variation in counts after pasteurization is within the limit of error inherent to the plate count. All of the slow acid formers were destroyed by pasteurization. Since the slow acid types constituted such a small percentage of the flora, the high dilution requisite for plating rendered it impossible to determine their numbers. Consequently, it is not known whether this group was destroyed by pasteurization or was not present in the original mix.

In all but one instance (180° F.), the rapid acid producers were completely destroyed, and the flora after pasteurization was divided between the alkali forming, inert, and peptonizing groups. The extract agar and caseinate agar counts were practically the same with these mixes.

It will be noticed that a very striking difference exists in the results obtained from pasteurizing mixes made of raw dairy products and mixes made of dairy products which had been previously pasteurized. A comparison of the data in tables 1 and 2 permits the following generalization:

1. Mixes made of raw ingredients showed a very high initial count, consisting mostly of rapid acid producers. After pasteurization these were uniformly reduced to sufficiently low numbers to permit plating in low dilutions. In these low dilution plates none of the slow acid producing types was observed. This suggests that they were either not present in the mix before pasteurization or had been destroyed by the heat.
2. In the mixes made from dairy products which had previously been pasteurized, lower initial counts were obtained as well as a lower efficiency of pasteurization. It was of special interest to note, however, that the flora after pasteurization consisted mostly of slow acid types. This suggests that these organisms were more resistant to heat when not grossly outnumbered by the rapid acid producing organisms. It is possible that the acidity produced by these organisms may have an effect on the thermal resistance of the cells.

Another possibility is that the slow acid formers were present in very small numbers in the raw products and constituted such a small percentage of the flora that they were immeasurable by the differential plate method. During pasteurization these products may become contaminated from the pasteurizing vat and other equipment. This possibility seems quite plausible in view of the fact that the slow acid producers usually survived pasteurization and would be expected to remain on the equipment unless complete sterilization was practiced. This contamination, together with the elimination of competitive types by pasteurization, would easily account for the high percentage of resistant types remaining in the mixes made from pasteurized ingredients.

Table 3 presents a summary of the average percentage of the various types of organisms before and after pasteurization for all the mixes in this experiment. In the raw mix the slow acid producers could not be determined because of the predominance of alkali forming colonies that rendered the entire plate alkaline, thereby obliterating the slow acid formers. The slow acid producing group and the alkali forming and inert groups combined constituted approximately 88 per cent of the flora of the raw mixes.

After pasteurization these two groups constituted roughly 97 per cent of the flora, of which approximately 75 per cent were slow acid formers. The observation that the flora after pasteurization consisted mostly of the slow acid formers is in keeping with the results of Ayers and Johnson (1)

TABLE 3

Summary of the percentage of types of organisms in the mix after pasteurization

PASTEURIZATION TEMPERATURE °F.	TIME IN MIN.	PER CENT OF TYPES			
		Acid formers		Peptonizers	Inert
		Rapid	Slow		
145	20	10.77	73.33	2.57	13.33
145	25	1.91	78.02	2.27	17.40
145	30	.25	77.19	2.77	20.29
150	20	.09	62.23	2.55	35.93
150	25	.02	64.60	3.02	32.36
150	30	.09	59.01	3.18	37.72
160	10	—	77.55	2.55	19.90
160	15	—	76.35	2.39	21.26
160	20	—	67.63	3.38	28.99
170	2	.09	79.12	2.46	18.33
170	5	—	77.57	2.72	19.71
170	10	—	74.88	2.73	22.39
180		—	70.89	2.76	26.35
Raw mix		11.55		1.11	

who found that 79 per cent of the bacterial flora of market milk after pasteurization consisted of acid formers.

With one exception the rapid acid producers were uniformly reduced to a small percentage. In only one mix, which was heavily contaminated with these organisms, did any of this group survive pasteurization. The peptonizers in all cases constituted only a minor portion of the flora both before and after pasteurization. A large portion of these organisms was destroyed by pasteurization although some survived all temperatures and time intervals employed.

EFFECT OF PASTEURIZATION UNDER PLANT CONDITIONS ON THE BACTERIAL FLORA OF THE MIXES

The results from the previous experiments were obtained under laboratory conditions, using test-tubes and flasks for pasteurizing equipment. Each of these experiments, however, was repeated with larger volumes of mixes, pasteurized under plant conditions, and the results obtained were essentially the same in all cases.

STUDY OF SLOW ACID FORMING ORGANISMS

Since the slow acid forming group comprised the majority of organisms resistant to pasteurization, further experiments were conducted with pure cultures of these types of bacteria.

It was observed that there were at least five distinct types of colonies present on the caseinate agar plates.

Morphological and biochemical study of 23 representative cultures revealed that they were Gram-positive rods which could be classified into three groups on basis of sugar fermentation. They grew well at 80° F. but not at 98° F., and although they grew very slowly litmus milk was eventually coagulated.

When a sterile mix was inoculated with pure cultures of these slow acid forming bacteria and held at 80° F., the rate of acid development as measured by the pH value was very slow. These organisms were found to be very resistant to heat. Temperatures of 170° F. for 10 minutes and 145° F. for 30 minutes did not materially reduce the count on mixes which had been inoculated with pure cultures.

SUMMARY

Standard ice cream mixes were made from raw dairy products and from ingredients which had been previously pasteurized. These mixes were pasteurized at various exposures and total and differential bacterial counts were made.

In the first trials pasteurization was carried out in test-tubes, but later, larger mixes were made and pasteurized under plant conditions to afford a comparison of the two methods.

It was found that none of the exposures employed for pasteurization yielded results sufficiently different to be measurable by the plate method. The results obtained by pasteurizing mixes under plant conditions were essentially the same as those obtained when pasteurization was done in test-tubes.

It was further found that in mixes made from raw dairy products the flora after pasteurization was about evenly divided between the peptonizing, and alkali forming and inert groups, whereas in mixes made from pasteurized ingredients the flora after pasteurization consisted largely of slow acid formers. The predominating types of these organisms were isolated and their characteristics determined.

The slow acid forming organisms isolated were small Gram-positive rods, which grew well at 80° F. but not at 98° F. Since they produce acid only slowly in ice cream mix, it is doubtful if they are of any significance in the souring of ice cream mixes.

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THE PHOSPHOLIPIDS IN MILK

I.—THEIR DISTRIBUTION AMONG SOME MILK PRODUCTS

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Although it has long been known that phospholipid-like compounds occur in milk, it was not until the early part of the present century that the approximate chemical nature of these substances was determined. Their exact chemical constitution is still in doubt. However, numerous attempts have been made to determine their approximate amounts in milk and in some of its products. A number of the results obtained by various authors are given in table 1. Only those publications which are most complete in their scope and in which the quoted results are most representative of those generally found in the literature have been cited in this table. Reference to other work has been given in a supplementary bibliography.

In a review of the literature pertaining to this subject Mohr and Moos (6) present an excellent bibliography and also summarize the results of the numerous authors cited as given in table 2.

TABLE 1

Phospholipids in Some Dairy Products According to Various Authors

AUTHOR	WHOLE MILK	SKIM MILK	CREAM	BUTTER	BUTTER-MILK	SEPARATOR SLIME
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Dornic & Daire (1)	.0595	.0332	.....	.....	.0651–.0944	.....
Glikin (2)	.0516 .1173	.0015	.....	.....	.....	.....
Bordas & Raczkowski (3)	.0909 .0252	.018	.334 (50%)*	.....	.....	.....
Hess & Helman (4)	.1819 .1377	.1072 .1156	.2668(32%)* .1859(20%)* .2668(32%)*	.....	.....	.....
Chapman (5)	.0345 .0709	.0082 .029	.1824 .2155	.....	.1036 .148	.....
Mohr & Moos (6)	.0038 .2889	.0015 .150	.05 .334	.014 1.6	.0332 .8768	.....
Schmidt & Muhlheim (7)	.0038	.....	.....	.153 .1736	.....	.....
Cusick (8)	.....	.....	.....	.0433 .0723	.....	.....
Rewald (9)	.05	.0096	.....	.95 1.40	.....	
Thurston & Petersen (10)	.....	.....	.....	.....	.36–.87	.....
Laxa (11)	.06	.....	.....	.....	.....	.2562 .3017
Grimmer & Schwarz (12)	.....	.....	.....	.....	.....	.419

* Fat content of cream.

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TABLE 2

Summary of the values reported by a number of authors for the phospholipid content of various dairy products (according to Mohr and Moos)

PRODUCT	MINIMUM AND MAXIMUM VALUES REPORTED
	per cent
Whole milk	.0038– .2889
Skimmilk	.0015– .15
Cream	.05 – .334
Butter	.014 –1.6
Buttermilk	.0332– .8768
Pasteurized milk	.1388– .1692
Milk fat	0–1.73

A discussion of the various methods employed by these authors is beyond the scope of this article. In most cases, however, the analyses were based upon the determination of the phosphorus content of isolated organic material with a subsequent calculation of this value as lecithin.

In several cases distearyl lecithin is assumed as the form present while in other cases other fatty acids are assumed as constituents of the molecule. It is now known that cephalin is also present in milk, but its exact chemical composition is not known. Hence, even in those cases wherein the values obtained are nearly equivalent they are not strictly comparable unless the same molecular weights have been used in the calculations.

Since the results reported vary so greatly, it seems probable that the cause therefor may be traced to the methods used in the isolation of the organic phosphorus-containing material.

In view of the wide discrepancies in the results noted and also in view of the fact that in no case reported are the results sufficiently complete to make possible the calculation of the percentage distribution of the phospholipid content of the original milk among its products, a complete study of this problem of distribution was initiated. The ultimate purpose of this study was to determine accurately the amount of phospholipids in the various products, which in turn would show to what extent they might introduce errors into the various tests used for the determination of the fat content.

EXPERIMENTAL

Method:

Since the work planned involved the determination of phosphorus upon small samples of fat, it was necessary to use a micro method. The method used was that described by Bloor (13), (14), (15), and by Kober and Egerer (16), as modified by Meigs (17), with the exception that a superoxyl free from phosphorus was used instead of nitric acid as an oxidizing agent in the digestion.

Preparation of samples:

The procedure when skimmed milk was used will serve as an example in describing the method. Three cc. of skimmed milk were run, drop by drop, into 80 cc. of a well agitated mixture of 3 parts of 95 per cent alcohol which had been redistilled from KOH, and 1 part of ether which had been distilled through alkaline pyrogallol to free it from peroxide. The mixture was then carefully heated to boiling in a water bath and held at that temperature for a short time; it was then cooled, made up to 100 cc. with an ether-alcohol mixture, and filtered. The solution was so aliquoted, on the basis of preliminary tests, that an amount was obtained adequate to give an accurate nephelometer reading with respect to the known standards used. This aliquot was placed in a 200×25 mm. pyrex boiling tube with a few glass beads, the tube partly immersed in a warm water-bath and the ether and alcohol carefully evaporated by gentle suction. One cc. of H_2SO_4 and 2 drops of 30 per cent superoxyl, free from phosphorus, were then added. The mixture was heated carefully over a micro burner, cooled, more superoxyl added as needed; and the procedure repeated until digestion was completed and a clear solution obtained. About 3 cc. of water was added and the solution brought to alkalinity with 20 per cent NaOH solution, and then a drop of 20 per cent HCl was added. This solution was then washed into a 25 cc. volumetric flask, a crystal of thymol added to prevent mold growth, and distilled water added to volume. This is the "unknown" solution.

Preparation of the standard solution:

The standard, or solution of known strength, was prepared by dissolving 0.6942 grams of KH_2PO_4 in distilled water and diluting this solution to 1 litre in a volumetric flask. Three and one-half, 7, 8, 9, 10, 11, 12, and 13 cc. respectively, of this solution were placed in 100 cc. volumetric flasks and each made up to volume. Preliminary tests were made with these solutions by placing 3 cc. of each in 25 cc. volumetric flasks, adding to each the exact amount of NaOH used in the unknown, neutralizing the solution with concentrated H_2SO_4 and making each up to volume, until the amount of solution best suited to the particular need at hand was ascertained.

Precipitating solution:

Fifteen grams of Merck's sodium molybdate was dissolved in 25 cc. of distilled water. To this 100 cc. of HCl of definite strength [see Meigs (17)] was added slowly during agitation and then 10 cc. of a 2 per cent solution of strychnine sulfate. This solution was allowed to stand over night in a dark place and then filtered through S & S No. 589 paper. A sufficient amount of this solution should be prepared at one time to last only through several days' work.

Precipitation:

Standards and "unknown" solutions should be precipitated in pairs with procedures as nearly identical as possible for each. Twenty-five cc. of distilled water are added to each of two 50 cc. volumetric flasks. Then 5 cc. of HCl of the same concentration as that used in making the precipitating solution are added and 5 cc. of the precipitating solution. During constant rotation of the flasks 10 cc. of the "unknown" solution are added to one flask and 10 cc. of the standard solution to the other. The content of each flask is brought to volume with water and mixed by inverting carefully 5 to 10 times. After at least 3 minutes the dispersion has formed and the solutions are ready for comparison. This is done in a Duboscq colorimeter modified for use as a nephelometer (15). Usually the average of 10–12 readings was used in calculating the percentages of phospholipids. The following equation was used:

$$\frac{\frac{\text{Reading of Standard soln.}}{\text{Reading of Unknown soln.}} \times \frac{\text{Wt. of phospholipids equivalent to}}{\text{Wt. of } KH_2PO_4 \text{ in known soln.}}}{\text{Wt. of substance used}} = \frac{X}{100}$$

X = percentage of phospholipids in unknown solution

To check the accuracy of the method the phospholipid content of egg lecithin was determined according to the method outlined and also by a macro-gravimetric method for the determination of phosphorus.

Calculation of the lecithin content from the phosphorus content determined gravimetrically gave a value of 2.65 per cent. Analyses of 8 replicate samples by the outlined nephelometric method gave an average lecithin content of 2.69 per cent (calculated as distearyl lecithin).

The phospholipid formula used:

According to Koch and Woods (18) the phospholipids of milk consist approximately of 55 per cent lecithin and 45 per cent cephalin. For our purpose we have assumed the presence of these constituents in equal amounts. According to Levene (19) lecithin contains one saturated, usually stearic, and one unsaturated fatty acid. Burow (20) believes, however, that the saturated acid in this case is butyric acid. Recent unfinished work in these laboratories has shown that the acids present in this compound are of relatively large molecular weights. Hence it has been assumed that the molecular weight of distearyl lecithin ($C_{44}H_{90}O_9NP = 807.76$) represents fairly accurately the average molecular weight of the lecithin in milk.

Levene states that cephalin is undoubtedly a stearo-linoleic amino ethyl alcohol ($C_{41}H_{78}O_8NP$), the molecular weight of which is 743.66.

The average molecular weight of these compounds (we have assumed their presence in equal quantities) is 775.71. The quantity of phosphorus represented by the dispersion in the standard used most frequently was

TABLE 3

The phospholipid content of milk and its products and the distribution of the phospholipids among the various products

PRODUCT		AMOUNT OF PRODUCT	FAT CONTENT	PHOSPHOLIPID CONTENT	TOTAL PHOSPHOLIPID CONTENT	DISTRIBUTION OF PHOSPHOLIPIDS	RECOVERY OF PHOSPHOLIPIDS
		lbs.	*per cent.*	*per cent.*	*lbs.*	*per cent.*	*per cent.*
Whole milk	(1)	324.0	2.55	.152	.492	100.0	
	(2)	276.0	3.80	.158	.435	100.0	
	(3)	246.0	4.34	.153	.376	100.0	
Cream	(1)	30.0	27.0	.224	.067	13.72	
	(2)	31.0	34.0	.269	.083	19.08	
	(3)	26.0	42.0	.284	.074	19.68	1. 95.67
							2. 91.79
Skimmilk	(1)	294.0	.07	.137	.403	81.91	3. 91.85
	(2)	245.0	.11	.129	.316	72.64	(slime included)
	(3)	220.0	.10	.123	.271	72.07	
Butter	(1)	8.9	86.5	.224	.020	4.06	
	(2)	12.2	85.7	.247	.030	6.90	1. 97.39
	(3)	12.2	85.5	.269	.033	8.78	
							2. 95.47
Buttermilk	(1)	19.6	.96	.284	.056	11.38	3. 96.11
	(2)	21.6	1.54	.318	.069	15.86	
	(3)	15.0	1.64	.382	.057	15.16	
Separator slime	(1)	18 g.	.....	.504	.0002	.041	
	(2)	25 g.	.....	.566	.00031	.071	
	(3)	23 g.	.....	.710	.00036	.096	

0.00001897 grams and represents therefore 0.0004742 grams of phospholipids.

The procedure outlined for use with skimmilk was used also for the determination of phospholipids in the other products. A number of trial runs were usually necessary to determine the amount of product most suitable for the determination.

Milk was obtained on three successive days and separated. The cream from milk No. 1 was kept in a sweet condition, that from No. 2 was inoculated with a butter starter and an acidity of 0.29 per cent allowed to develop. The cream from No. 3 was also inoculated and an acidity of 0.58 per cent allowed to develop. The creams were then churned and the butters washed in the usual manner but not salted. At each stage of separation of the products samples were taken, and the amount of each product was weighed. The amount of phospholipid in each sample was then determined in the manner described. Complete data on these samples are given in table 3.

DISCUSSION

The variations first noted in the values upon the three samples are in the relative amounts of phospholipids in the three creams. In sample No. 1, which was skimmed to give a 27 per cent fat content in the cream, the phospholipid content was 13.72 per cent of the total amount in the whole milk while the other two samples of greater fat content contained relatively more phospholipids. The skimmilk from the latter two samples contained proportionately smaller amounts of this constituent than that from sample No. 1.

As indicated in table 3 the phospholipid content of the butters increased with increases in the fat content of the cream. Approximately 30 per cent of the total phospholipid content of cream from sample No. 1, 36 per cent of that from sample No. 2, and 45 per cent of that from sample No. 3, passed over into the butter in the churning process.

The results reported here for the phospholipid content of whole milk and skimmilk check well with the results obtained in these laboratories on other samples of these products.

The values obtained with the creams are perhaps a trifle low. This seems to be indicated by the percentage-recovery figures obtained when the values for skimmilk and cream are totaled, as well as by the fact that the value for cream is, in every case, less than the combined values for its respective butter and buttermilk. The greatest divergence between other results obtained in these laboratories and those reported here is found in the values reported for buttermilks.

The average phospholipid content of commercial buttermilks, which will be reported later, is approximately 0.27 per cent, several samples showing greater amounts. The seemingly high values reported here may perhaps be

partly accounted for by the fact that the water used to wash the butter was added to the buttermilk and the phospholipid content calculated on the basis of the weight of the original buttermilk. With increases in the fat content of the cream there is an increase in its phospholipid content. On the basis of the results reported in this paper the phospholipid percentages found in the literature for the various products are, with but few exceptions, low. The results of Dornic and Daire (1) indicating that cream contains approximately twice as much phospholipids on a percentage basis as does its respective milk are in agreement with this work.

Because of the great variations in fat content in the creams used, no definite conclusion can be drawn concerning the effect of different acidities upon the phospholipid distribution between the butter and buttermilk. However, the effect of acidity appears small, and therefore fishiness, when it does develop, is probably due more to other factors than to the increased amount of phospholipids which is incorporated into the acid cream butter.

The amount of phospholipids in the various products is of such magnitude that it can be of significance in fat determinations of whole milk, skimmilk, and buttermilk. In the case of whole milk, this is true only if the test used removes totally, with the fat, the phospholipids present. Moreover, the normal variations in the values obtained with the various tests upon whole milk are greater than the value represented by the phospholipid content. Hence, the amount of phospholipids may be of significance only in the testing of skimmilk and buttermilk.

SUMMARY

A micro-nephelometric method has been used in the determination of the phospholipid content of whole milks and their respective skimmilks and creams. The creams were churned and the phospholipid content of their respective butters and buttermilks was determined by the same method. The percentage distribution among its various products of the phospholipids present in whole milk has been calculated in each case from the amount of product obtained and its determined phospholipid content.

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II.—THE PHOSPHOLIPIDS IN SKIMMILKS AND THEIR EFFECT UPON THE ACCURACY OF THE VARIOUS FAT TESTS

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The results reported in the preceding paper indicate that the phospholipid content of skimmilk is approximately 0.13 per cent. On the assumption that the fat content of this product is 0.10 per cent, the maximum possible error that could be introduced in a fat test if practically all of the fat and phospholipids were removed would be approximately 100 per cent.

Thurston and Peterson[1] have attempted to determine approximately the amount of phospholipid in buttermilk by the difference in the percentage of fat obtained by the gravimetric (extraction) method and that obtained by the Babcock method. Their proof of the validity of the method was based on the results obtained with these two methods applied to synthetic skimmilk to which various amounts of fat and lecithin had been added. The difference in the results obtained by the two methods was approximately equal to the amount of lecithin added.

In order to prove the reliability of their method of procedure the validity of several assumptions need to be established. First, it would have to be proved that the naturally occurring phospholipids are totally removed by the extraction method, as was the added lecithin. This is not the case, for though the added lecithin can be completely extracted by the Roese-Gottlieb method, as indicated in their work, the naturally occurring phospholipids, as will be shown later, are extracted only to the extent of 20 per cent or less of their total amounts. Secondly, it must be assumed that the value obtained with the use of the Babcock method is a fairly accurate measure of the fat content of a skimmilk. It is a well-known fact that all of the fat is not removed by this method. It will be shown later that in skimmilks of 0.10 per cent or less fat content, less than 25 per cent of the fat is removed by the Babcock method. Furthermore, aside from the inefficiency of these fat tests, the variations which normally occur invalidate the use of these tests for even approximating the amounts of phospholipids in skimmilk, which are present to the extent of only 0.13 per cent.

The purpose of the present investigation was to determine the extent to which the phospholipids of skimmilk accompany the fat in the various tests and the magnitude of the error thus introduced.

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[1] Thurston, L. M., and Peterson, W. E. Lipins and sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods. JOUR. DAIRY SCI. 11: 270–83. 1928.

TABLE 1

The phospholipid and fat content of skimmilk and the phospholipid content of the fat removed by various tests

SAMPLE NO.	PHOSPHOLIPIDS IN SKIMMILK	FAT TEST				PHOSPHOLIPID CONTENT OF FAT				FAT CONTENT—CORRECT READINGS			
		Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb	Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb	Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb
	per cent.	*per cent.* *	*per cent.* *	*per cent.* *	*per cent.* †	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
1	.13	.12	.20	.22	.21	trace	—	trace	6.67	.12	.20	.22	.20
2	.12	.02	.05	.07	.10	"	—	"	16.51	.02	.05	.07	.08
3	.13	.02	.04	.06	.11	"	—	"	16.22	.02	.04	.06	.09

* Average of 8 duplicate determinations.

† Average of 4 duplicate determinations.

EXPERIMENTAL

Liquid skimmilk:

Three samples of liquid skimmilk were analyzed for fat by four different methods, namely, Roese-Gottlieb, Babcock, Modified Babcock (butyl alcohol method) and the Minnesota Babcock (alkaline reagent). A number of replicate determinations were also made in Babcock whole milk test bottles in the regular manner prescribed for each Babcock test. The fat is each case was siphoned off, washed with water until practically neutral in reaction, dried, and weighed. The phospholipid content of the fats isolated in this manner and of those isolated by the Roese-Gottlieb method was determined by the method described in the previous paper. The results are given in table 1.

The most consistent and the highest results were obtained by the Roese-Gottlieb method. This, however, was the only method that removed any appreciable amount of phospholipids. When corrections are made for the amount of the material contained in each fat the results obtained with this test are still greater in value than those obtained with other tests, although the results obtained with the use of the Modified Babcock test check well with these values in 2 of the 3 cases. The recovery of fat by the Minnesota Babcock method was not so great as the recovery by these two methods. The Babcock method recovered only a small amount of the fat present.

Dry skimmilk:

Five samples of spray-dried skimmilk were reconstituted on the basis of 10 grams in 100 cc. of water.

Their fat contents were determined by the four methods used in the case of liquid skimmilk. Extractions of fat from each sample with various tests were made as previously described and the phospholipids in these fat samples determined. The results are given in table 2.

In these tests, as in those with liquid skimmilk, the Roese-Gottlieb method removes the largest amount of fat, which contains approximately 10 per cent of phospholipids. The Modified Babcock method again gives results that compare favorably with those of the Roese-Gottlieb method, although they are not as consistent. Results with the Minnesota Babcock vary to a greater extent from those obtained with the Roese-Gottlieb method than did analogous results upon liquid skimmilk. On the assumption that the values obtained by the Roese-Gottlieb method—corrected for the phospholipids present—are the correct ones for the fat content of the product, the relative amounts of fat recovered by the other methods are given in table 3.

DISCUSSION

The assumption that the Babcock method gives even an approximately correct value for the fat content of skimmilk is untenable. The further assumption previously mentioned, that the extraction method removes all

TABLE 2

The phospholipid and fat content of dry skimmilk and the phospholipid content of the fat removed by various tests.

SAMPLE	PHOSPHOLIPID CONTENT		FAT CONTENT				PHOSPHOLIPID CONTENT				CORRECT READINGS			
	Soln.* basis	Dry basis	Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb	Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb	Babcock	Minn. Babcock	Mod. Babcock	Roese-Gottlieb
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	.104	1.15	trace	.05	.08	.09	trace	.....	trace	9.94	trace	.05	.08	.081
2	.101	1.12	"	.06	.08	.10	"	.....	"	11.13	"	.06	.08	.089
3	.101	1.12	"	.04	.05	.08	"	.....	"	14.49	"	.04	.05	.068
4	.085	.94	"	.03	.05	.07								
5	.09	.99	"	.04	.08	.10								

Fat tests = average of 4 duplicate determinations.

* Soln. 10 grams dry skimmilk in 100 cc. of water.

TABLE 3
Comparison of the amount of fat recovered by the various methods

SAMPLES		FAT CONTENT	RECOVERY OF FAT — % OF ROESE-GOTTLIEB		
		Roese-Gottlieb* = 100 %	Modified Babcock	Minn. Babcock	Babcock
			%	%	%
Liquid skimmilk	(1)	.196	110	105	60
	(2)	.083	88	52	24
	(3)	.092	62	46	22
Dry skimmilk	(1)	.081	92	57	Less than 10
	(2)	.089	87	68	" " "
	(3)	.068	70	54	" " "
	(4)	.07 (not corr.)	69	43	" " "
	(5)	.10 (not corr.)	85	40	" " "

* Corrected for phospholipid content.

of the phospholipids under practical conditions of procedure, is likewise proved untenable by the results presented in tables 1 and 2. Hence the difference in values obtained by these two methods does not give the true phospholipid content of skimmilk.

A correlation of the results presented here with those of Thurston and Petersen indicates clearly that the lecithins added to a milk are not incorporated in the state in which they exist in the natural product.

With respect to the tests and their accuracy when used with skimmilks, the results show that the greatest recovery of fat is obtained when the Roese-Gottlieb test is used. Due to phospholipids also extracted with this method the values obtained may be in error to the extent of 15 per cent when the fat content of the skimmilk is approximately 0.10 per cent. In the case of higher fat contents the error is proportionately less. Results obtained with the Modified Babcock test check well with those obtained with the extraction method in most cases, but the latter method seems to give the most consistent results. Results obtained with the Minnesota Babcock test are low in value, especially in the case of the dry skimmilks.

SUMMARY

The phospholipid content of skimmilk was found to be approximately 0.13 per cent.

The Babcock, Modified Babcock, and the Minnesota Babcock methods remove only negligible amounts of these compounds. The extraction method (Roese-Gottlieb) removes phospholipids to the extent of 15 per cent of the fat in skimmilk containing approximately 0.10 per cent fat, which is equivalent to approximately 12 per cent of the total amount of the phospholipids present in the milk. Since the amount of phospholipds extracted from various milks by the Roese-Gottlieb procedure is approximately constant, the percentage of error in determining the fat content by this method becomes less with increases in the fat content of the sample and *vice versa*.

III.—THE PHOSPHOLIPIDS IN BUTTERMILK AND THEIR EFFECT UPON THE ACCURACY OF VARIOUS FAT TESTS

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Previous work has indicated that the phospholipid content of buttermilks will vary with the fat content of the cream used in the churning. Buttermilk from cream of approximately 27 per cent fat contained 0.284 per cent phospholipids; that from 34 per cent cream 0.318 per cent; and that from 42 per cent cream 0.382 per cent.

The experiments of Thurston and Petersen (1) wherein they determine the amount of lecithin added to synthetic skimmilk, by the difference between the values obtained by the gravimetric and Babcock methods, respectively, have been discussed in the previous paper. These investigators have further applied the same method to the determination of the phospholipid content of four buttermilks from creams of different fat content. The results obtained vary from 0.36 per cent in the case of buttermilk from cream of 22 per cent fat to 0.87 per cent in buttermilk from cream of 68 per cent fat content. The objections to this method of procedure, cited in the previous paper, are even greater when the procedure is applied to buttermilk.

Chapman (2) has studied the recovery of lecithin, added to buttermilk, by the Babcock, Modified Babcock, and Extraction (Mojonnier) methods. The Babcock method recovered amounts equivalent to from 14.3 to 157.4 per cent of the added lecithin. The Modified Babcock method recovered amounts equivalent to from 16.2 to 105.6 per cent, and the extraction method recovered amounts equivalent to from 49.3 to 93 per cent of the added lecithin. With the extraction method the average recovery in 16 samples was 76.5 per cent, hence the author increased the value for the lecithin content of the extracted material (calc. from the phosphorus content) by a multiple equivalent to the ratio of 100/76.5. The assumption that 76.5 per cent of the phospholipids in buttermilk is extracted under practical conditions of testing is not valid, as will be shown by the following experiments.

EXPERIMENTAL

Eight samples of buttermilk were obtained from the Grove City, Pennsylvania, creamery and their fat contents determined by the Babcock, Modified Babcock, and Roese-Gottlieb methods. By procedures similar to those used in testing, the fat columns resulting in the Babcock and Modified Babcock methods were extracted with ether, washed, and dried. The phospholipid content of this fat was determined as described in a previous paper. The results are given in table 1.

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TABLE 1

The phospholipid and fat content of liquid buttermilk and the phospholipid content of the fat removed by various tests

SAMPLE NO.	PHOSPHOLIPIDS IN BUTTERMILK	FAT CONTENT				PHOSPHOLIPID CONTENT OF FAT			FAT CONTENT—CORRECTED VALUES		
		Babcock†	Modified Babcock†	Minn. Babcock	Roese-Gottlieb‡	Babcock	Modified Babcock	Roese-Gottlieb	Babcock	Modified Babcock	Roese-Gottlieb
	%	%	%	%	%	%	%	%	%	%	%
1	.224	.70	.99		.75	trace	trace	9.58	.70	.99	.68
2	.258	.42	.75		.63	.031	.141	11.72	.42	.75	.56
3	.325	.37	.83		.60	.052	.038	10.72	.37	.83	.54
4	.275	.30	.65		.53	.040	.051	12.01	.30	.65	.47
5	.280	.09	.45		.34	.180	.094	19.75	.09	.45	.27
6	.180*	.16	.55		.44	.048	.303	11.64	.16	.55	.39
7	.174*	.08	.38		.33	trace	.203	12.90	.08	.38	.29
8	.186*	.05	.42		.28	"	.114	17.12	.05	.42	.23
A			.96	.73							
B			1.54	1.52							
C			1.64	.86							

* Results low.

† Average of 12 duplicate determinations.

‡ Average of 4 duplicate determinations.

Though relatively greater amounts of phospholipids were extracted from buttermilk by the Babcock and Modified Babcock tests than were extracted from the skimmilk samples in the previous work, the amounts were so small that practically no corrections of the original values obtained were necessary. As in the case of skimmilk, the Roese-Gottlieb test removes appreciable amounts of phospholipids. From 8 to 17 per cent of the fat was shown to be phospholipids, the percentage depending upon the amount of fat in the original buttermilk. The relationship between the fat content of a buttermilk sample and the percentage of phospholipids in the fat extracted by the Roese-Gottlieb test is given in Fig. 1.

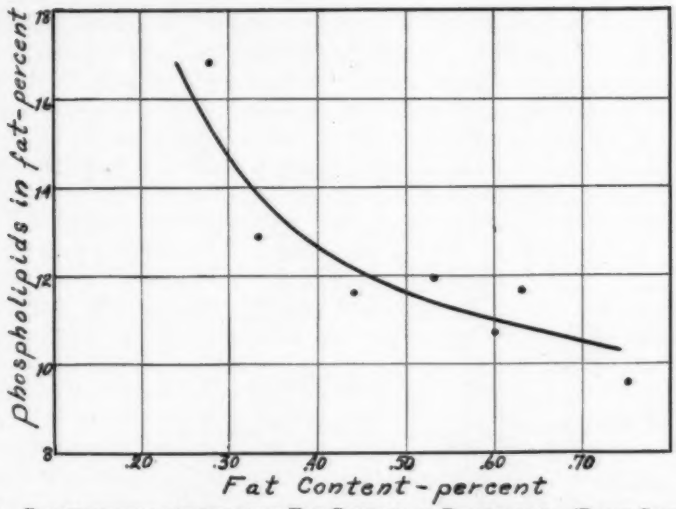

FIG. 1. RELATIONSHIPS BETWEEN THE FAT CONTENT OF BUTTERMILKS (ROESE-GOTTLIEB) AND THE PHOSPHOLIPID CONTENT OF THE EXTRACTED FAT.

As the fat content of the buttermilk decreases, a greater percentage of the extracted material is phospholipids. Hence the error due to the presence of these compounds increases with decreases in the fat content and *vice versa*.

In view of the fact that the Minnesota Babcock test was not used in these experiments with liquid buttermilks, three other samples were obtained and their fat contents determined by this test and by the Modified Babcock method. The results obtained have been included in table 1. These results are analogous to those obtained upon skimmilks with these tests, namely, that the Modified Babcock recovers more of the fat than the Minnesota Babcock method, and agree with those obtained by Lyons and Finlay (3), who used the Gerber method, a procedure very similar in principle to the Modified Babcock method.

TABLE 2

The phospholipid and fat content of dry buttermilks and the phopholipid content of the fat removed by various tests

SAMPLE NO.	PHOSPHOLIPID CONTENT OF DRY BUTTERMILKS		FAT CONTENT				PHOSPHOLIPID CONTENT OF FAT				FAT CONTENT—CORRECTED VALUES			
	Dry basis	Sol'n. basis	Babcock*	Minn. Babcock†	Modified Babcock‡	Roese-Gottlieb§	Babcock	Minn. Babcock	Modified Babcock	Roese-Gottlieb	Babcock	Minn. Babcock	Modified Babcock	Roese-Gottlieb
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
S_1	1.54	.140	.07	.19	.63	.54	.02	trace	.034	13.16	.07	.19	.63	.47
S_2			.06		.51	.45								
S_3			.12		.49	.43								
S_4			.16		.56	.49								
S_5	1.88	.170	.02	.16	.40	.41	.01	trace	.026	17.23	.02	.16	.40	.34
R_1			.32		.645	.52								
R_2			.26		.53	.49								
R_3	1.84	.167	.37	.43	.93	.75	.01	trace	.015	7.16	.37	.43	.93	.70
R_4			.09		.53	.49								
R_5	1.82	.165	.09	.17	.51	.44	.054	trace	.022	14.16	.09	.17	.51	.38

* Average of 8 determinations.
† " " 4 "
‡ " " 8 "
§ " " 4 "

Dry buttermilks:

Five spray-dried and five drum-dried samples of buttermilk were reconstituted and analyzed for their fat contents by the Babcock, Modified Babcock, Minnesota Babcock, and Roese-Gottlieb tests. Procedures similar to those used in each test were followed on a large number of samples and the fat in each case isolated as described previously. This fat was then analyzed for its phospholipid content by the micro-nephelometric method. Because of the difficulties of reconstituting the roller-dried samples a small amount of ammonia was added in each case and the samples stirred until the solutions seemed homogeneous. The results obtained are given in table 2.

The results are similar to those obtained with liquid buttermilk, except that smaller amounts of phospholipids were removed by each test. In all except the Roese-Gottlieb test the percentages removed with the fat were so small that no correction of the original reading was necessary. In case of the results obtained with the Roese-Gottlieb test a correction of 6 to 15 per cent, depending upon the fat content of the original buttermilk was necessary. The total phospholipid content of dry buttermilk seems low in value. Whether this is due to the destruction of phospholipids or to incompleteness of extraction is not known.

TABLE 3

Comparison of the amount of fat recovered by the various tests

PRODUCT	FAT CONTENT ROESE-GOTTLIEB (CORR.) = 100%	RECOVERY OF FAT PER CENT OF ROESE-GOTTLIEB		
		Modified Babcock	Minn. Babcock	Babcock
Liquid buttermilks	%	%	%	%
1	.69	143	.....	100
2	.55	136	.....	76
3	.53	156	.....	70
4	.47	113	.....	64
5	.27	126	.....	33
6	.38	145	.....	42
7	.27	141	.....	30
8	.23	182	.....	22
Dry buttermilks				
1	.48	131	40	15
2	.35	114	46	6
3	.70	133	61	53
4	.39	130	43	23
Liquid buttermilks (Actual fat content)				
A	.....	.96	.73	.....
B	.....	1.45	1.52	.....
C	.....	1.64	.86	.....

On the assumption that the values obtained by the Roese-Gottlieb method —corrected for the phospholipids present—are correct for the amount of fat in the product, the relative amount of fat removed from liquid and dry buttermilks, on a percentage basis, is as shown in table 3.

DISCUSSION

Of the fat tests used only the extraction method removed appreciable amounts of phospholipids. The amount removed by the Roese-Gottlieb procedure was equivalent to from 6 to 17 per cent of the fat extracted or 30 per cent of the total amount of phospholipids present in the buttermilk.

These results dispute the validity of the procedure used by Thurston and Petersen, who assumed that since lecithin added to synthetic skimmilk can be recovered almost completely by the gravimetric method but not by the Babcock method, the amount of phospholipids in buttermilk could be determined approximately by the difference between the results obtained by the application of these two methods to this latter product. That the Babcock method does not give a true estimate of the fat and that the extraction method does not remove all of the phospholipids is evident from the results presented.

By the Modified Babcock (butyl alcohol) method Thurston and Peterson recovered both the fat and the lecithin added to synthetic skimmilk. On the basis of these results they state "the results reported in this paper, however, are considered by us to show beyond question that the use of this method should be discontinued." It is evident from the data presented in this paper that the quoted statement was made on the basis of erroneous data since under practical conditions the butyl alcohol method removes only traces of the phospholipids.

In view of the results obtained by Thurston and Petersen and by Chapman, Petersen and Herreid (4) they state that "the results obtained by Thurston and Petersen (1928) and later confirmed by Chapman (1928) showed that the Roese-Gottlieb, the Mojonnier (a commercial adaptation of the Roese-Gottlieb method), and the normal butyl alcohol methods do not give an accurate estimate of the true fat content of buttermilk. This discrepancy was shown to be due to the fact that buttermilk contains relatively large amounts of ether and alcohol soluble phospholipids of which lecithin and cephalin are the principal constituents." They state further "having definitely established the status of the problem, it became evident that a test was needed whereby a true fat content of buttermilk could be accurately estimated." With this thought as a basis Petersen and Herreid modified the alkaline reagent methods, tried by numerous workers, for use in the testing of buttermilks. To test the efficiency of the reagent finally adopted they used the same methods of procedure which were used in the work of Thurston and Petersen, namely, recovery of added lecithin and fat. That conditions produced when lecithin is added to milk do not simulate those

existing in the natural product must be concluded from a consideration of their experiments and those reported herein. Hence, the method of proof used in their work does not reflect the true efficiency of each method in recovering fat and lecithin-like substances from the natural product.

The results which we have presented in this paper indicate that in the testing of buttermilk the extraction and Modified Babcock methods are the most efficient for the removal of fat and of these the Modified Babcock method removes a greater amount than the extraction method. The average difference in the case of the liquid buttermilks amounts to about 40 per cent of the value obtained with the extraction method—corrected for its phospholipid content. Though it is known that part of the fat recovered with the Modified Babcock method is due to the presence of some extraneous material in the fat column this large difference in values can not be accounted for in this manner. The results obtained upon dry buttermilks are analogous to those with liquid buttermilk except for the fact that the differences between those obtained by the Modified Babcock method and the extraction method are of less magnitude. With dry buttermilks as with liquid buttermilks the recovery of fat by the Minnesota-Babcock test was found to be unsatisfactory, amounting to approximately one-half or less of that removed by the Roese-Gottlieb method (values corrected for phospholipid contents).

SUMMARY

The phospholipid content of eight sweet cream commercial buttermilks was found to be approximately 0.27 per cent. No appreciable quantities of phospholipids are recovered in the fat when the Babcock, Modified Babcock, or Minnesota-Babcock methods are used, but when the extraction method (Roese-Gottlieb) is used corrections for the presence of these compounds to the extent of from 6 to 17 per cent of the values obtained, depending upon the fat content of the sample, must be made.

More fat is recovered from liquid and reconstituted dry buttermilks when the Modified Babcock (butyl alcohol) method is used than is recovered by any of the other methods tried.

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FACTORS GOVERNING THE MANUFACTURE OF SWEET-CURD COTTAGE CHEESE[1]

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Recently so-called sweet-curd cottage cheese has appeared on the market in many localities. A number of methods for the manufacture of this product have been offered, most of which require the use of rennet extract, doubtless on the assumption that, because rennin can cause coagulation at acidities far below those necessary for acid coagulation, a low-acid curd will result.

The literature on the subject of cottage cheese manufacture, though voluminous, is concerned mostly with setting forth various directions for making the product and places little or no emphasis on the factors governing flavor and body characteristics. However, Van Slyke and Hart (5) found that, in the "cooking" process, heating cottage cheese curd of the acid-coagulated type higher than 100° F. made it too hard and dry, and that the proper water content was about 75 per cent. Recent work has consisted largely of searching for satisfactory methods of making cottage cheese with the use of rennet extract. In studying this process Thurston (2) found that if cutting takes place at a whey acidity lower than 0.41 per cent, calculated as lactic acid, an over-firm or rubbery curd usually results. This finding was confirmed by Wilson and Trimble (4) except that they report the limit below which a tough curd results as 0.45 per cent.

Goss and Mutten (1) have reported a method of making sweet-curd cottage cheese without the use of rennet extract.

EXPERIMENTAL

Although 243 batches of cheese were manufactured in conducting this work, data from many batches made when the work was begun were of little value due to a lack of knowledge of the factors concerned, which resulted in failure to control conditions so that the factors could be studied separately. Consequently, data from 146 batches are reported in this paper. Each batch of cheese was made in a 50-gallon cheese vat so as to approximate commercial conditions as nearly as possible, approximately 300 pounds per batch being used.

Soon after this work was begun it became evident that the production of curd of a sweet, desirable flavor was not difficult, being dependent on the

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removal of free whey from the curd by sufficient firming and by careful washing after draining. The production of curd of desirable firmness and texture, in the form of flakes of uniform shape and size, proved to be difficult, and this study therefore was concerned mostly with finding the factors that govern the production of curd of desirable body and texture characteristics.

When these experiments were begun, so little was known of the factors governing the character of curd made with rennet extract that a method which had been used with some success was adopted and studied. This method consisted of pasteurizing skimmilk at 185° F. for 20 minutes, cooling to 70° F., and adding lactic culture, 25 per cent calcium chloride solution, and rennet extract, in varying quantities. These variations per thousand pounds of skimmilk ranged as follows: lactic culture, 0.4 to 13 pounds; calcium chloride, none to 2.66 ml.; and rennet extract, 1.3 to 3.3 ml. The milk was set in the evening and was ready to cut the following morning after a coagulation period of 14 to 16 hours. The curd was cut when it showed a "clean break" over a thermometer raised through the curd while held at an angle nearly horizontal. Horizontal cutting was done with a knife having blades one-half inch apart, and vertical cutting with a knife having blades one inch apart. Flat flakes resulted large enough to present the desired flaky appearance in the finished curd, but small enough to facilitate "cooking." It was soon found that high temperature cooking was necessary for curd made by this method. Consequently the average final temperature was 152° F. This is a much higher temperature than other workers have recommended and, as will be shown later, it was required because of the high temperature used in pasteurizing the skimmilk. The curd resulting under these conditions was very soft at the time of cutting, and great care was necessary to avoid breaking and shattering of the flakes during the early stages of the cooking process. Heat was applied slowly and the curd was stirred only after little or no breaking of the flakes results. The cooking process was usually completed in two or three hours. The whey was drained and the curd washed with cold water, drained, and trenched. The curd was then sampled and observations as to its characteristics were recorded. This cooking procedure is referred to later as Method 1.

In the use of the cooking procedure outlined above an attempt was made to produce curds of like firmness and texture from all batches. Because this proved impossible, a carefully controlled method of treatment after cutting the curd, which would permit direct comparisons between batches, was necessary. To meet this requirement heating of the jacket was started slowly immediately after cutting the curd, but stirring was not started until 10 minutes after cutting in order to allow the freshly cut surfaces to

"heal." Just before the first stirring of the curd approximately 30 pounds of water at a temperature of 140°–150° F. was run over the curd so as to increase the temperature more rapidly than was possible by heating from the jacket alone. Stirring and heating then followed to produce a steady increase of temperature up to 120° F., which was reached in every case within a few minutes of one-half hour after cutting. During this heating period observations were recorded of the rapidity with which whey was expelled from the curd, amount of shattering of curd flakes, and progress of firming of the curd. At the end of this half-hour period a sample of curd was placed on a 20-mesh screen, chilled for 5 minutes in ice-water, and drained for 10 minutes on the screen over a funnel with occasional stirring to facilitate draining. At the end of the draining period the screen was tapped gently against the funnel to remove the loose water, and the texture, firmness, and flavor of the curd was observed, after which it was analyzed for moisture content.[2]

The vat temperature was maintained at 120° F. for 30 minutes. This was done to determine whether maintenance of temperature or increase in temperature produced the greater effect on the curd. At the end of this holding period the curd was sampled and analyzed in the same manner as at the end of the first 30-minute period. During the third 30-minute period the vat temperature was increased from 120° F. to 150° F. at as uniform a rate as possible, and with sufficient stirring to transfer the heat uniformly throughout the curd and whey. At the end of this heating period the curd was sampled and analyzed as before. Next followed a 20-minute holding period at 150° F., after which a fourth sample was taken and analyzed. The whey was then drained and the curd trenched. This procedure is referred to later as Method 2.

Method 2 was used to study the effects of the temperature at which the skimmilk was pasteurized, the setting temperature, the varying quantities of rennet extract, and the varying whey acidities at cutting on the type of curd produced. It also yielded a determination of the effect of cooking procedure on the firming process.

[2] Moisture determinations were made in duplicate as follows: 10 grams of thoroughly mixed and mashed sample were weighed into a flat dish 15 cm. in diameter. When slightly less than 10 grams were in the dish the curd was spread evenly over the bottom of the dish and then more cheese was added to bring the weight to 10 grams. Weighing was done on balances designed for butter analysis. The samples were dried in an oven at 100° C. to constant weight. In weighing the dried samples it was necessary to place a 6-gram weight in the dish to aid in reaching a balance, as the moisture content of the cheese exceeded the limit of the measurements provided on the beams of the scales. Six grams amounted to 60 per cent moisture and the percentages shown by the scale riders after a balance was reached were added to 60 to give the percentage of moisture in the curd.

Effect of Acidity of Whey at Time of Cutting

A study of the data from 91 batches using rennet extract in skimmilk pasteurized at 185° F. and cooked according to the procedure outlined as Method 1 showed that the acidity of the whey at the time of cutting governed the firmness and texture of the curd to a greater extent than any other factor or combination of factors. The acidity was determined by titration of 17.6 ml. samples (approximately 18 grams) immediately after cutting, and is recorded in terms of per cent lactic acid. The data are presented in table 1 in the form of three frequency distributions, the first

TABLE 1

Distributions of whey acidities showing the relation of acidity of whey at the time of cutting to the production of curd of desirable firmness

ACIDITY	FIRM—GOOD CURD	SLIGHTLY RUBBERY CURD	RUBBERY CURD
	Number of cases	Number of cases	Number of cases
0.20–0.245			4
0.25–0.295			2
0.30–0.345		3	
0.35–0.395	9	3	
0.40–0.445	11	1	
0.45–0.495	30		
0.50–0.545	13		
0.55–0.595	13		
0.60–0.645	1		
0.65–0.695	1		
Total	78	7	6

being a grouping of all batches from which firm, desirable curd resulted, the second, a grouping of batches from which slightly rubbery curd resulted, and the third, distinctly rubbery curd. At cutting, the average whey acidity of those batches which produced desirable curds was 0.48 per cent, and the majority[3] of these acidities was in the range of from 0.41 to 0.54. A slightly rubbery or a rubbery curd resulted almost invariably when, coagulated under the above conditions, it was cut at whey acidities lower than 0.41 per cent, whereas the curd was almost certain to be rubbery when cut at acidities lower than 0.35 per cent.

The curd produced when cutting was carried out at a whey acidity of 0.35 per cent or lower behaved in cooking as a rennet curd, while that cut at acidities higher than 0.41 per cent behaved like acid curd. The former matted quickly in spite of considerable stirring, and whey was expelled from the curd rapidly, whereas the latter did not mat, but was soft and difficult to handle during the first stages of cooking.

[3] Determined by the formula $M_a \pm \sigma$.

TABLE 2

Data on batches from skimmilks pasteurized at 145° F., made with or without rennet extract, using a setting temperature of 90° F.

BATCH NO.	CC. RENNET EXTRACT PER 1000 LBS.	WHEY ACIDITY AT CUTTING	MOISTURE CONTENT AT INTERVALS OF				*FIRMNESS AT INTERVALS OF				REMARKS
			½ hr.	1 hr.	1½ hrs.	2 hrs.	½ hr.	1 hr.	1½ hrs.	2 hrs.	
180	0	0.52	81.80	82.00	72.55	73.45	2	3	4	4	Matted, broken and shattered some
192	0	0.52	80.30	82.45	77.20	77.80	3	3	4	4	Matted, shrunken, dry
182	0	0.54	81.7	82.15	75.05	73.80	3	3	4	4	Matted, shrunken, dry
208	0.75	0.41	81.3	82.2	77.55	74.70	3	3	4	4	Matted, broken, shrunken
221	0.75	0.45	78.15	77.30	75.10	76.00	2	3	4	4	Not heated to 150 but to 130. Matted, broken
210	0.75	0.49	83.75	82.55	80.60	78.65	2	2	4	4	Matted, shrunken
201	0.75	0.53	83.60	80.30	80.50	80.60	2	2	3	3	Failed to record observations
202	0.80	0.56	84.25	82.75	79.40	80.20	2	2	3	3	Matted slightly. No breakage of flakes
235	1.0	0.42	78.80	78.80	73.65	72.40	3.5	3.5	4	4	Matted, broken, dry
223	1.0	0.48	82.15	81.75	78.90	78.05	3	3	3.5	3.5	Matted, slightly shattered
209	1.0	0.50	83.10	83.40	79.55	78.85	2	2.5	3.5	4	Matted slightly, no breaking or shattering
203	1.0	0.55	83.4	83.70	80.70	81.40	2	2.5	3	3	Matted some. Shattered on heating
225	1.25	0.48	80.4	81.5	77.8	78.25	2	3	3.5	4	Matted slightly. Shattered and broken
204	1.25	0.52 alkali off	82.95	83.00	81.70	81.65	2.5	2.5	3	3	Matted slightly, but split where cut. No shattering
213	1.25		80.0	79.0	76.15	74.85	2	2	3.5	3.5	Matted, shrunken
212	1.25	0.57	83.2	81.15	80.7	77.65	2	2	2.5	3	Matted slightly, but split where cut. No shattering

TABLE 2.—*Continued*

BATCH NO.	CC. RENNET EXTRACT PER 1000 LBS.	WHEY ACIDITY AT CUTTING	MOISTURE CONTENT AT INTERVALS OF				*FIRMNESS AT INTERVALS OF				REMARKS
			½ hr.	1 hr.	1½ hrs.	2 hrs.	½ hr.	1 hr.	1½ hrs.	2 hrs.	
		%									
238	1.5	0.43	75.00	77.25	74.20	75.36	3	3	4	4	Matted, but split where cut, no breaking or shattering
226	1.5	0.47	82.90	84.40	80.85	80.75	2	2.5	3	3	Slightly matted, but split on cut line. Slightly broken, shattering
205	1.5	0.54	82.40	82.60	81.70	81.60	2	2.5	3	3	Slightly matted, but split on cut line. No shattering
216	1.5	0.54									Shattered and broken, so could not be finished
229	1.75	0.44	82.25	81.15	75.95	76.25	3	3	4	4	Same as above
239	1.75	0.445	80.50	79.125	76.40	75.10	3	3	4	4	" " "
206	1.78	0.56	84.40	84.50	81.20	82.10	2	2.5	3	3	Not matted, slightly shattered, curd crumbly
231	2.0	0.47	80.09	80.20	76.15	75.35	3	3	4	4	Matted considerably but mostly split where cut
207	2.0	0.54									Shattered and broken, so could not be finished
240	2.0	0.415	78.45	78.90	74.40	74.50	3	3	4	4	Matted slightly but split where cut. No shattering
241	2.0	0.44	80.30	81.10	78.05	77.75	3	3	3.5	3.5	Matted slightly split where cut, shattered slightly
242	2.25	0.44	81.40	81.75	77.70	77.30	2.5	2.5	3.5	3.5	Matted slightly, but split where cut, shattered slightly
243	2.5	0.445	80.85	81.25	79.10	79.12	2.0	2.0	2.5	2.5	No matting, shattered and broken
181	3.3	0.48	81.60	80.60	79.30	79.25	2	3	3.5	3.5	Matted slightly, no breaking or shattering
182a	3.3	0.58									Shattered and broken, could not firm

* 1. Soft, mushy curd.
2. Fairly soft curd.
3. Firm, desirable curd.
4. Over-firm, dry and shrunken, or rubbery curd.

It was observed during this study in comparing batches set under identical conditions and in which the same amount of rennet extract was used, that, when cutting occurred at relatively high whey acidities, the firming of the curd took place at a slower rate than at lower acidities. A study of the data in table 2 confirms this observation. Furthermore this effect is strikingly apparent in the results from batches 214 and 215 shown in table 3. Setting conditions for these batches were identical except for an insignificant variation in the amount of rennet extract used, but batch No. 215 which was cut at a whey acidity of 0.51 per cent firmed more rapidly and was firmer when finished than No. 214 which was cut at a whey acidity of 0.63 per cent.

In practical cottage cheese manufacture, when rennet extract is used, the common practice of cutting the curd when it shows a "clean break" over the finger should be abandoned and the acid test substituted, inasmuch as two coagulants are being used: *i.e.*, rennin and acid, the curds of which cannot be differentiated by the finger test. Furthermore the acidity has been shown here to be a factor in the control of the manufacturing process.

The Effect of Setting Temperature

The cooking procedure already described as Method 1 was used first in these trials. The amount of culture used was 0.5 to 1 per cent of the amount of skimmilk used. It was found that when the setting temperature was changed from 70° to 90° F. and the amount of culture increased to 5 to 10 per cent of the amount of milk used, the coagulating period could be shortened 50 per cent or more, making it possible to manufacture a batch of cottage cheese in 4 to 6 hours, whereas 14 to 16 hours were required when a setting temperature of 70° was used.

The cooking procedure previously described as Method 2 was applied to a number of batches set at 70° F. The data from 9 representative batches made in this way are shown in table 3. They may be compared to data shown in table 2 from batches for which a setting temperature of 90° F. was used. A significant fact shown by these results is that the amount of rennet extract required in batches set at 70° F. is considerably greater than in batches set at 90° F. It appears that it is desirable to use 4 to 5 ml. of rennet extract per thousand pounds of skimmilk in batches set at 70° F. whereas 1 to 1.5 ml. are all that may be used safely at 90° F.

Observations of the batches described above indicate that the setting temperature of 90° F. is preferable to 70° F. The use of the higher setting temperature reduced the time required for completing the process so that the entire procedure could be carried out during the normal working day. This means that, in general, a more accurate control of the process is possible than when 70° F. is used, since the maker can be on hand throughout the entire setting period, and can be certain to cut the curd when the

TABLE 3

Data from representative batches for which a setting temperature of 70° F. was used

BATCH NO.	CC. RENNET EXTRACT PER 1000 LBS. SKIM	WHEY ACIDITY AT CUTTING	*FIRMNESS OF CURD AT INTERVALS OF			
			½ hr.	1 hr.	1½ hrs.	2 hrs.
211	1.0	0.60	2	2	2.5	2.5
214	1.25	0.63	1	2	2.5	2.5
215	1.28	0.51	3	3	4	4
220	1.5	0.60	1	2	2.5	3
222	2.0	0.52	1	2	2.5	2.5
225	3.0	0.56	2	2	2	2
227	4.0	0.55	1.5	2	2	2.5
228	5.0	0.55	2	2	2	2
230	6.0	0.50	Shattered, failed to firm			

*1. Soft, mushy curd.
2. Fairly, soft curd.
3. Firm, dry, desirable curd.
4. Over-firm, dry, shrunken or rubbery curd.

proper acidity has been reached. Furthermore, it was observed that fermentations which produced undesirable off-flavors occurred more frequently at the lower temperature than at the higher.

The Effect of Pasteurizing Temperature

Pasteurizing temperatures and holding times of 145° F. for 30 minutes, 165° F. for 30 minutes, and 185° F. for 20 minutes were used in this work. Setting temperatures were 90° F. Rennet extract was not used in some of the batches. The cooking procedure designated as Method 2 was used throughout.

Fifteen batches were made in studying the effects of pasteurizing temperatures on the process of manufacturing cottage cheese. Observations showed that pasteurization at the two higher temperatures caused a reduction of the firming properties of the curd after coagulation whether or not rennet extract was used. For this reason it was necessary to cook to higher temperatures to produce desirable firmness in the curd when the skimmilk used had been pasteurized at 165° and 185° F. than when pasteurization was at 145° F.

It was also observed that curd from skimmilk pasteurized at the two higher temperatures lost some of its cohesive properties. This effect was evident from the fact that when the curd from skimmilk pasteurized at the two higher temperatures was first stirred after cutting, it showed a decided tendency to shatter, whereas this tendency was not observed in curd from skimmilk pasteurized at 145° F. In some further studies these general

effects of the temperature of pasteurization were confirmed in the case of acid curds (3). The tendency toward shattering was undesirable because it resulted in the formation of irregularly shaped flakes of curd which are objected to in many markets. Furthermore, much of the fine grained curd shattered from the flakes is lost in the whey at draining. The shattering and breaking of curd from skimmilk pasteurized at the two higher temperatures usually resulted in a reduction of the size of the flakes so that they doubtless firmed at a faster rate than would have been possible had there been no breakage.

Pasteurization at 145° F. for 30 minutes favored the formation of a curd which did not shatter on stirring unless excessive quantities of rennet extract were used. The effect of varying quantities of rennet extract will be discussed later. The curd firmed more readily on heating and was less prone to become crumbly than were the curds from skimmilks pasteurized at the two higher temperatures.

The Effect of Amount of Rennet Extract

On the basis of the foregoing results, which showed that the whey acidity at the time of cutting must be 0.41 per cent or higher, that a setting temperature of 90° F. produces better results than 70° F., and that pasteurization of skimmilk should be carried out at 145° F. for 30 minutes, it was possible to narrow down the study. The next step was to determine the effects of varying quantities of rennet extract under the setting conditions outlined above, and with the cooking procedure previously described as Method 2. Batches were made using no rennet extract, followed by those using rennet extract at the rates of 0.75, 1.0, 1.25, 1.5, 1.75, 2, 2.25, 2.5, and 3.3 ml. per 1000 pounds of skimmilk. Whey acidities at the time of cutting were varied through as definite a range as possible for each level of rennet extract.

Data from the batches made under these conditions are summarized in table 2. Under the rather rapid cooking conditions used, those batches in which no rennet-extract was used firmed rapidly. These curds also showed a marked tendency toward matting immediately after heating was begun, and although the matting was reduced somewhat by cutting at the higher whey acidities, it was evident and troublesome nevertheless. The matting of the flakes made it necessary to break them by stirring, and as the curd was rather tender at this stage, the flakes generally did not break along the lines where they had been cut. As a result, the flakes were not uniform in shape or size after matting ceased, as it usually did after a temperature of 115° to 125° F. was reached in cooking.

When rennet extract was used at the rate of 0.75 ml. per 1000 pounds of skimmilk, matting was reduced slightly, and as the quantity of rennet extract was increased, matting was markedly reduced. Quantities of 1.5

ml. rennet extract and higher, in combination with relatively high whey acidities at cutting, tended to cause shattering of the curd on stirring during the early stages of cooking. In some of the extreme cases the curd failed to show any cohesive properties at all, but disintegrated, becoming fine grained. In these cases coagulation by rennin had occurred an hour or more before the desired acidity was reached. It seems likely that the development of acid after coagulation by rennin changed a large part, or all, of the calcium paracaseinate to free paracaseinate which lacked cohesive properties. These results indicate that rennet extract must be used at a rate such that it will not produce coagulation very far in advance of the development of the proper acidity for cutting.

These changes suggest the true function of rennin in this type of cheese. Its coagulating properties are of secondary importance while its important function is to prevent matting of the curd flakes which, when matted, must be broken, resulting in unsightly, irregular-shaped flakes prone to be rubbery and unpalatable.

To produce the most desirable curd the proper balance must be obtained between rennin action and acid development. As much rennet extract as possible should be used in order to reduce matting to a minimum. The amount to be used based on the observations made in these experiments will be determined by the strength of the extract and the cutting acidity selected, and is the amount just below that which causes curd disintegration on stirring. For standard, fresh rennet extract the amounts per 1000 pounds of skimmilk for the various acidity ranges are as follows: 0.41–0.45 per cent acid, 1.75 cc.; 0.46–0.50, 1.5 cc.; 0.51–0.55, 1.25 per cc.; 0.56 upward, 1.0 cc.

When a setting temperature of 70° F. was used a marked increase in the amount of rennet extract was necessary to produce the beneficial effects of rennin as shown by a comparison of the data in tables 2 and 3. At 70° F. about 4 to 5 ml. of rennet extract per thousand pounds of skimmilk were found to be the most desirable amounts. Batches in which less rennet extract was used showed a tendency to mat during the early stages of cooking, whereas batches in which 6 ml. were used were prone to shatter excessively. It should be pointed out that the whey acidity at cutting for each of three of the first four batches recorded in table 3 was high. This explains why these batches failed to firm sufficiently. On the other hand batch No. 215 which was cut at a whey acidity of 0.51 per cent firmed and matted excessively because not enough rennet extract was used. This is in direct contrast to batch 230 cut at slightly lower acidity which failed to firm at all because of the use of an excessive amount of rennet extract.

These results were obtained from the use of fresh rennet extract. When a rennet extract weakened by age was used the amount had to be increased.

The Effect of Temperature to Which Curd is Heated in the Whey

The cooking procedure referred to as Method 2 permitted a study of the effect of cooking temperature on the firming of the curd. The effects of whey acidity at cutting, setting temperature, temperature at which the skimmilk is pasteurized, and the amount and strength of rennet extract on the firming of the curd have already been discussed. By studying the curd at different stages of the uniform cooking procedure it was possible to determine to what temperature each curd should have been heated. The data included in table 2 yield information as to the proper cooking procedure for each given condition when skimmilks are pasteurized at 145° F.

In general it is seen that desirable firmness usually was produced at temperatures between 120° and 150° F. It was observed that a marked shrinking of the curd occurred in these batches at about 140° F., which narrows the range in which desirable curd results to 120° to 140° F. Temperatures lower than 120° F. were usually not high enough to produce a dry curd such as is desired by the sweet curd trade. Furthermore, the whey remaining in the curd tended to cause an acid flavor. Within the range from 120° to 140° F. the proper finishing temperature will depend on coagulating conditions as already shown, and on whether the market being served desires a relatively soft or a very firm curd. Firming progressed rapidly while the temperature was being increased, but more slowly during the two holding periods. In cases when cutting occurred at relatively high whey acidities, and also in many cases when relatively large amounts of rennet extract were used, little or no firming occurred during the periods when the temperature was kept constant. It is indicated, therefore, that cooking should consist of a steady increase of temperature until the curd is firmed to the proper degree.

The effect of firming is produced largely by the expelling of moisture from the curd. The coefficient of correlation between moisture content and firmness was found to be $-0.74 \pm .046$. The effect is also shown by the regression line, using moisture as the independent and firmness as the dependent variable, which will be found in figure 1. It should be pointed out that the moisture data are relative, but that the moisture contents of the curds treated as previously described are higher than they would have been had a longer time been allowed in each case for the drainage of free water. It is believed that the moisture results are directly comparable, however, because a uniform procedure was followed in all cases. The moisture content of the finished curd after draining in the vat for one hour was usually about 2 per cent lower than that of the same curd, after it had been sampled, chilled, and drained under the conditions described as part of Method 2.

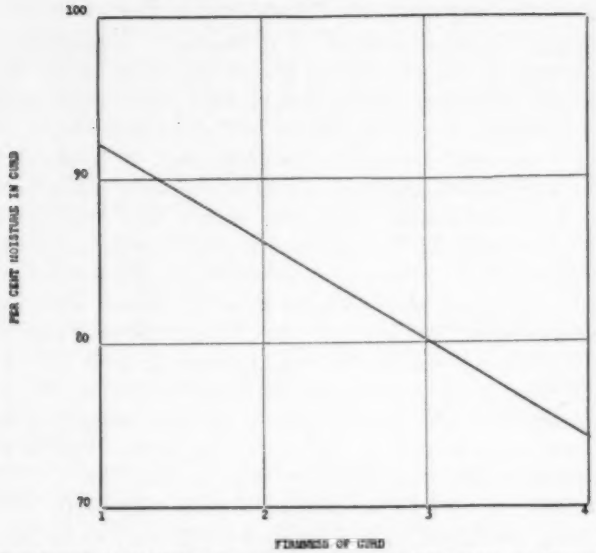

FIG. 1. REGRESSION LINE SHOWING THE RELATION OF FIRMNESS OF COTTAGE CHEESE CURD TO ITS MOISTURE CONTENT.

The Effect of Calcium Chloride in Batches in Which Rennet Extract Was Used

Calcium chloride is known to stimulate rennin action and was, therefore, used during the early part of these experiments in all but five of the batches made by Method 1. No differences could be noted between the batches in which calcium chloride was used and those from which its was omitted. The negligible effect of calcium chloride is probably explained by the fact, already established, that coagulation by rennin is not desirable before acidity has developed to a point sufficiently high to insure the production of desirable body and texture characteristics of the curd. So little rennet extract is required to produce this effect that the use of calcium chloride solution in the strength and quantities employed in this work had no noticeable effect.

The Effect of Quantity of Culture Used

In using the coagulating conditions found by these experiments to produce the best results: *i.e.*, development of whey acidities of 0.41 per cent or higher, setting at 90° F., using the skimmilks pasteurized at 145° F., and small quantities of rennet extract, the addition of culture at the rate of 5 to 10 per cent of the quantity of skimmilk gave more desirable results

than smaller inoculations because acid development was more rapid than when smaller amounts were used. In this work 10 per cent culture was used mostly, but 5 per cent culture, without changing the amount of rennet extract gave similar results. Culture inoculations lower than 5 per cent are not desirable because of the longer time required to develop the necessary amount of acid.

The relation of per cent culture inoculation to the rate of acid development was determined for skimmilks pasteurized at 145° F. and held at

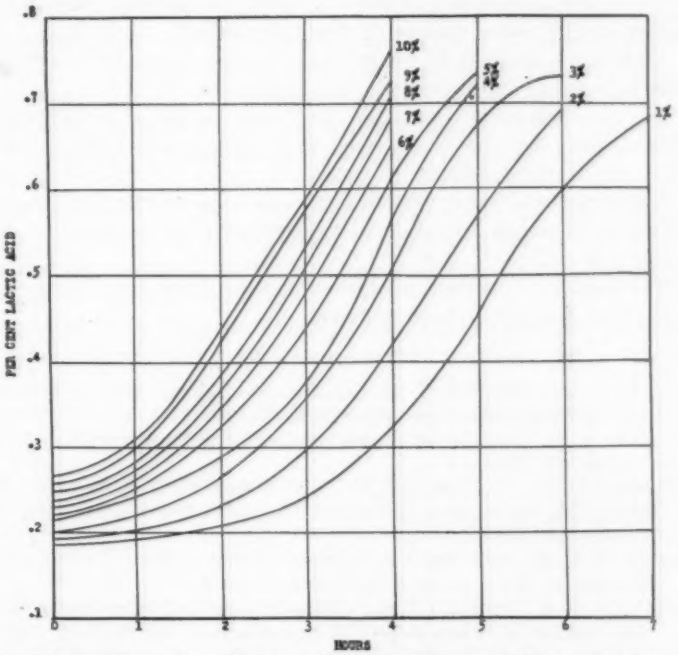

FIG. 2. A COMPARISON OF THE RATES OF ACID DEVELOPMENT IN SKIMMILKS INOCULATED WITH 1 TO 10 PER CENT LACTIC CULTURE AND INCUBATED AT 90° F.

90° F. Three trials were made, in two of which fresh culture was used and in one of which culture held for two days at approximately 40° F. was used. The results were averaged to yield the data plotted in figure 2.

CONCLUSIONS

1. When rennet extract is used as a coagulant in the manufacture of cottage cheese the acidity of the whey at the time of cutting must be 0.41 per cent or higher to prevent excessive matting and the production of a hard rubbery curd on cooking.

2. A setting temperature of 90° F. is preferable to 70° F. because at the higher temperature the time required for coagulation is reduced, and fermentations causing off-flavors are less prone to develop than at 70°.

3. Pasteurization of the skimmilk to be used for cottage cheese manufacture should be carried out at 145° F. for 30 minutes rather than at higher temperatures, when a holding period is used, because the resulting curd firms more readily and is less prone to shatter and break than when higher pasteurizing temperatures are used.

4. The important function of rennet extract is to prevent matting of the curd after cutting rather than to produce coagulation.

5. Excessive quantities of rennet extract cause excessive shattering of the curd on stirring and retard the firming process.

6. The temperature to which the curd must be heated in the whey to produce uniform firmness is governed by coagulating conditions and the acidity of the whey at the time of cutting.

7. Under the conditions obtaining in these experiments the use of calcium chloride in setting the milk had no apparent effect on coagulation and curd production.

8. The quantity of culture used in setting should be 5 to 10 per cent of the amount of skimmilk to be coagulated.

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THE ESCHERICHIA–AEROBACTER GROUP OF BACTERIA IN DAIRY PRODUCTS[1]

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INTRODUCTION

The *Escherichia-Aerobacter* group comprises one of the important groups of bacteria in dairy products. Milk, even under careful conditions of production, practically always contains some of these organisms. The fact that the *Escherichia* type generally represents organisms coming from the intestinal tract of man and animals and the *Aerobacter* type organisms from soils and grains makes a distinction between the two types useful.

The growth of *Escherichia-Aerobacter* organisms in dairy products is undesirable for, in addition to forming acid and gas from lactose, they produce undesirable flavors and aromas. Some of the defects which have been reported as due to this group of organisms are ropiness in milk and cream, and gassy fermentations in cottage cheese, cheddar cheese, milk, and sweetened condensed milk.

Review of the literature shows that most of the work on the *Escherichia-Aerobacter* group of bacteria in dairy products has been done with types which, in many cases, have not been defined as species. Levine (9) has summarized the incidence of Aerobacter types among colon bacilli isolated from milk by six investigators as follows:

Investigators	*Number of strains studied*		*Per cent Aerobacter section (V.P. + M.R. −)*
Orr* (13)	850		39.0
MacConkey (10)	26		57.8
Rogers, Clark & Davis (14)	124		47.5
Hulton (4)	18		72.3
Wood (20)	93		17.5
Stokes (16)	271		59.8
Total	1,382	Average	43.1

* Glucose fermenters, lactose reaction not recorded.

These investigators found that strains belonging to the *Aerobacter* section ranged between 17.5 and 72.3 per cent. Based upon the weighted

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average of all the strains studied (1,382 strains), 43.1 per cent belonged to the *Aerobacter* section.

Kline (7) found that 35 per cent of 407 cultures isolated from milk belonged to the *Escherichia* section, 51 per cent to the *Aerobacter* section and 14 per cent to an intermediate section.

The systems of classification used in the past have varied so with respect to characters used for identification of species that it is difficult to compare the work of different investigators. Recognition of species has also been rendered difficult in some cases by incomplete descriptions.

Hunter (5) studied 590 cultures isolated from 135 samples of market milk. The cultures were grouped according to MacConkey's (10) classification, and were classified according to origin by their reaction to methyl red. Of 295 cultures identified as *Escherichia communior* (*B. coli communior*) 74.5 per cent gave an acid and 25.7 per cent an alkaline reaction to methyl red, and of 279 cultures identified as *Aerobacter aerogenes* (*B. lactis aerogenes*) 55.5 per cent gave an acid and 44.4 per cent an alkaline reaction. Hunter did not describe the technique used in the methyl red test so that it is impossible to compare his results with the results obtained in the present study where the methyl red test was used in conjunction with the Voges-Proskauer reaction to separate members of the genus *Escherichia* from members of the genus *Aerobacter*.

METHODS

Gentian violet lactose bile broth was used as an enrichment medium. This medium was used because of the work of Kessler and Swenarton (6) who found that the formation of gas in gentian violet lactose bile when inoculated with milk or diluted milk was a positive indication of the presence of the *Escherichia-Aerobacter* group which for practical purposes needed no confirmation.

The gentian violet lactose bile dehydrated medium had the following composition:

Bacto oxgall	10	parts
Bacto peptone	10	"
Bacto lactose	10	"
Gentian violet	0.04	"

Thirty grams of the above medium were dissolved in 1,000 cc. of distilled water. The resulting solution was tubed in Durham fermentation tubes and sterilized in the autoclave at 15 pounds pressure for 20 minutes.

Fermentation tubes were inoculated in duplicate with dairy products diluted in multiples of 10. Gas formation was recorded after 48 hours incubation at 37° C. The approximate number of *Escherichia-Aerobacter* organisms per cc. was determined by the dilution method from an adaption

of McCrady's (12) tables as given by Buchanan and Fulmer (2) for the most probable number of organisms with two tubes of each dilution.

Eosin methylene blue agar plates were streaked from tubes of the highest and lowest dilutions showing gas after 24 hours' incubation. Streaks were made from tubes incubated for 24 hours instead of 48 hours in order to lessen the possibility of one type overgrowing another and in order to lessen the possibility of certain types being inhibited or destroyed by acid formation. The possibility of drawing erroneous conclusions from eosin methylene blue agar streaks from the presumptive test has been well shown by the work of Ruehhoft, Kallas, Chinn and Coulter (15) with the Chicago Sanitary Board. They found that it was possible for either *Escherichia coli* or *Aerobacter aerogenes* to overgrow the other in lactose enrichment when both were initially present in equal numbers so that distorted views of the initial condition were obtained.

A direct plating method was used for the isolation of cultures, whenever possible. It was used in preference to the enrichment method in order to overcome objections already stated. Eosin methylene blue agar was used for the plating medium due to the work of Levine (9), who found that *Escherichia coli* and *Aerobacter aerogenes* could be correctly designated from their appearance on this agar in about 90 per cent of the cases. Typical well isolated colonies of *E. coli* on eosin methylene blue agar were from two to three mm. in diameter; neighboring colonies showed little tendency to run together; colonies were slightly raised with the surface flat or slightly concave, rarely convex; centers of colonies were dark and almost black, extending more than three-fourths across the diameter of the colony; colonies were dark, button-like, often concentrically ringed with a greenish metallic sheen.

Typical well isolated colonies of *A. aerogenes* were larger than those of *E. coli*, usually from four to six mm. in diameter or more; neighboring colonies ran together quickly; colonies were considerably raised and markedly convex with the center occasionally dropping precipitately; centers of colonies were deep brown, not as dark as those of *E. coli* and smaller in proportion to the rest of the colony; striated internal structure was often observed in young colonies; colonies were much lighter than those of *E. coli* with a metallic sheen not observed except occasionally in the depressed center when such was present.

Preliminary study showed that it was usually possible to differentiate *Escherichia* from *Aerobacter* types of surface colonies when milk or cream was plated directly with eosin methylene blue agar in dilutions of 1:10 or higher. Surface colonies could be successfully differentiated into types by macroscopic examination. Eosin methylene blue agar plates with all of the colonies at the surface were secured by placing the inoculum on the surface of pre-dried agar plates and then distributing it uniformly over

the surface with a glass rod bent at a right angle. When all of the inoculum was absorbed upon the surface of the agar the plates were inverted and incubated for 48 hours at 37° C. The number of colonies of each type was then recorded and a representative colony of each type isolated for pure culture study. Plates were often unsatisfactory for isolation purposes due to the presence of large numbers of other types of colonies or because of too few or too many colonies caused by the wrong dilution. In these cases, cultures were isolated by the enrichment method instead of by the direct plating method.

Colonies representing each of the *Escherichia* and *Aerobacter* types present were picked from the eosin methylene blue agar plates to standard agar slopes. Cultures were replated and colonies repicked three times before identification studies were started.

TESTS FOR IDENTIFICATION

Tests used for identification of cultures were the methyl red and Voges-Proskauer tests, utilization of citrate as the sole source of carbon, motility, liquefaction of gelatin, reduction of nitrates, formation of indol, action on litmus milk and fermentation of glucose, lactose, sucrose, dulcitol and salicin, with acid and gas production.

Methyl red test and Voges-Proskauer reaction.—The medium used had the following composition:

Glucose	0.5	gram
Proteose-Peptone (Difco)	0.5	"
Dibasic potassium phosphate	0.5	"
Distilled water	100	cc.

Ten cc. of the inoculated medium were incubated for 5 days at 30° C. and then divided into two equal portions. For the methyl red test, 5 drops of methyl red solution were added to 5 cc. of the medium. A positive reaction was indicated by a distinct red color and a negative reaction by a yellow color. The methyl red indicator solution was prepared by dissolving 0.1 gram of methyl red in 300 cc. of 95 per cent alcohol and diluting to 500 cc. with distilled water.

The Voges-Proskauer reaction was carried out according to Werkman's (18) modification. Two drops of a 2 per cent solution of $FeCl_3$ were added and then 5 cc. of a 10 per cent solution of NaOH. The mixture was shaken and allowed to stand for a short time when a permanent copper color appeared in the case of a positive reaction.

Utilization of citrate as the sole source of carbon was determined by the presence or absence of growth in Koser's citrate medium after 3 days' incubation at 37° C. The medium had the following composition:

Sodium ammonium phosphate	1.5	gram
Monobasic potassium phosphate	1.0	"
Magnesium sulphate	0.2	"
Sodium citrate	3.0	"
Distilled water	100	cc.

Motility was determined in one per cent glucose broth after 18 to 20 hours' incubation at room temperature.

Gelatin liquefaction was determined in a gelatin medium incubated for 14 days at 37° C. and then cooled in ice-water to show the presence or absence of liquefaction. The medium used had the following composition:

Beef extract	0.3	gram
Peptone	0.5	"
Gelatin	12.0	"
Distilled water	100	cc.

Nitrate reduction was determined in a medium of the following composition:

Beef extract	0.3	gram
Peptone	0.5	"
Potassium nitrate	0.1	"
Distilled water	100	cc.

The medium was incubated for 4 days at 37° C. A distinct pink or red coloration when a few drops of sulphanilic acid and alphanapthylamine reagents were added to the broth culture indicated nitrate reduction.

Indol formation was determined by the Góre technic as described in the "Manual of Methods for the Pure Culture Study of Bacteria" (11). The test was performed in a one per cent solution of tryptophane broth which was incubated for 3 days at 37° C.

Litmus milk was incubated for 14 days at 37° C. Color reaction, extent of coagulation, ropiness and extent of peptonization were observed.

Fermentation of carbohydrates was observed in a broth composed of one per cent peptone and one per cent of the test carbohydrate. A 5 per cent alcoholic solution of brom-cresol-purple was added to the broth as an indicator at the rate of 0.5 cc. per liter of medium. Gas production was observed in Durham fermentation tubes incubated for 3 days at 37° C. All carbohydrate broths were sterilized for 20 minutes at 15 pounds pressure with the exception of lactose broth which was sterilized for 12 minutes at 12 pounds pressure to lessen the chances of inversion. Sterility of the lactose broth tubes was determined by holding the tubes over-night at 37° C.

DATA

In the study reported herein, 204 cultures belonging to the *Escherichia-Aerobacter* group were identified on a species basis according to the scheme

of classification used in Bergey's (1) Manual. The cultures were isolated from raw milk, pasteurized milk, raw cream, ice cream, ropy milk and cream, and from defective butter.

Raw Milk

Fifty raw milk samples from individual patrons of the Iowa State College pasteurizing plant and 20 samples from the composite supplies of 11 other pasteurizing plants in Iowa were studied. Standard agar plate counts of the milk supplied the Iowa State College pasteurizing plant ranged between 1,700 per cc. and 5,000,000 per cc. with 76 per cent of the counts less than 500,000 per cc. Standard agar plate counts of the raw milk from the 11 other pasteurizing plants ranged between 52,000 and 75,000,000 per cc. with only 30 per cent of the counts less than 500,000 per cc.

The number of *Escherichia-Aerobacter* organisms in the raw milk supplied to the Iowa State College pasteurizing plant ranged from less than 1 per cc. to 25,000 per cc. with 53 per cent of the samples containing less than 10 organisms per cc. In the case of the 11 other pasteurizing plants, the number of *Escherichia-Aerobacter* organisms ranged from less than 1 per cc. to 250,000 per cc. with only 15 per cent of the samples containing less than 10 organisms per cc. Sixty per cent of the samples contained over 100 *Escherichia-Aerobacter* organisms per cc.

Ninety-one cultures were isolated from 70 samples of the raw milk. Thirty-seven per cent of the cultures were isolated by the direct plating method and 63 per cent by the enrichment method. Sixty-three per cent of the cultures belonged to the genus *Escherichia*, 26 per cent to the genus *Aerobacter* and 11 per cent to the intermediate group (Table 1). Cultures belonging to the intermediate group gave a positive or intermediate methyl red test, a negative Voges-Proskauer reaction and utilized citrate as the

TABLE 1

Percentage of cultures belonging to the genus Escherichia, the genus Aerobacter and an intermediate group

DAIRY PRODUCTS	NUMBER OF		PER CENT OF CULTURES IN THE		
	Cultures	Samples	Genus *Escherichia*	Genus *Aerobacter*	Intermediate Group*
Raw milk	91	70	63	26	11
Pasteurized milk	21	64	57	10	33
Raw cream	42	24	33	57	10
Ice cream	16	20	31	56	13
Ropy milk and cream	9	9	0	100	0
Defective butter	25	25	0	88	12

* Methyl red +, Voges-Proskauer −, Utilization of citrate +.

TABLE 2

Percentage of cultures belonging to the different species of the Escherichia-Aerobacter group

SPECIES	DAIRY PRODUCTS INVESTIGATED					
	Raw milk (91 cultures)	Pasteurized milk (21 cultures)	Raw cream (42 cultures)	Ice cream (16 cultures)	Ropy milk or cream (9 cultures)	Defective butter (25 cultures)
E. coli	13	10	10	13		
E. pseudocoloides	10	24		6		
E. communior	9	5	7	13		
E. paragrünthali	9	10	5			
E. vesiculiformans	7	5				
E. formica	4		5			
E. enterica	4		2			
E. anaerogenes	3		2			
E. grünthali	2	5				
E. neapolitana	1		2			
A. aerogenes	10		31	19	67	60
A. cloacae	11	10	14	38		12
A. oxytocum	5		12		33	16
Intermediate group*	11	33	10	13	0	12

* Methyl red +, Voges-Proskauer −, Utilization of citrate +.

sole source of carbon. They corresponded to the intermediate section described by Koser (8) who found organisms of this type to be widely distributed in unpolluted soil. Werkman and Gillen (19) proposed the generic name *Citrobacter* for this group and have described 7 species belonging to this genus. No attempt was made in this study to identify the cultures of the intermediate group on a species basis.

Kline (7) found that 34 per cent of 157 cultures isolated from pre-pasteurized milk belonged to the *Escherichia* section, 57 per cent to the *Aerobacter* section and 9 per cent to the intermediate section. He found approximately the same relationship for Grade A raw milk and for Certified milk.

Ten species were found of the genus *Escherichia* (Table 2): *Escherichia coli* constituted 13 per cent of the cultures; *E. pseudocoloides* 10 per cent; *E. communior* 9 per cent; *E. paragrünthali* 9 per cent; *E. vesiculiformans* 7 per cent; *E. enterica* 4 per cent; *E. formica* 4 per cent; *E. anaerogenes* 3 per cent; *E. grunthali* 2 per cent and *E. neapolitana* 1 per cent.

Three species were found of the genus *Aerobacter*. *Aerobacter aerogenes* constituted 10 per cent of the cultures; *A. cloacae* 11.0 per cent; and *A. oxytocum* 5 per cent. Other species may have been present but not in sufficient numbers to be detected by the isolation methods employed.

Kline (7) identified 92 cultures isolated from milk (presumably raw milk) on a species basis. He found 4 species in the *Escherichia* section while the author found 10. The same species were encountered in both studies with the exception of *Escherichia acidilactici* which was found by Kline but not by the author. Kline found that *E. neapolitana* was the species most common in the *Escherichia* section while the author found it to be the least common species.

In the *Aerobacter* section Kline found *Aerobacter aerogenes* and *Aerobacter cloacae*, and the author found in addition to the above a lesser number of cultures of *Aerobacter oxytocum*. *Aerobacter aerogenes* and *Aerobacter cloacae* are probably the two species of the *Aerobacter* section most commonly encountered in milk.

Pasteurized Milk

Thirty-nine samples of pasteurized milk from the Iowa State College pasteurizing plant and 25 samples from 13 other pasteurizing plants in Iowa were studied. The samples were taken from the pasteurizing vat, from the cooler inlet and from the bottled pasteurized product. The presence of organisms belonging to the *Escherichia-Aerobacter* group in the pasteurized milk did not, therefore, necessarily indicate survival of pasteurization but may have been due to subsequent contamination. Thirty per cent of the pasteurized milk samples gave positive tests for the presence of *Escherichia-Aerobacter* organisms. The counts ranged from less than

1 per cc. to 250 per cc. Due to the small number of *Escherichia-Aerobacter* organisms in the positive samples, all of the cultures had to be isolated by the enrichment method.

Twenty-one cultures were isolated from 19 positive samples. Eleven of the positive samples were collected at the Iowa State College pasteurizing plant of which 10 were from the bottled pasteurized milk while 1 was from milk in the pasteurizing vat immediately following pasteurization. Of the remaining 8 positive samples taken at 6 other pasteurizing plants, 3 were from bottled milk while 5 were taken direct from milk in pasteurizing vats. Examination of temperature recording charts showed that in 3 of the 5 cases where positive results were obtained directly after pasteurization, the milk was pasteurized either below 142° F. or else was not held for a 30 minute period.

Fifty-seven per cent of the 21 cultures isolated from the pasteurized milk belonged to the genus *Escherichia*, 10 per cent to the genus *Aerobacter* and 33 per cent to the intermediate group (Table 1). Kline (1930) found that 58 per cent of 50 cultures isolated from pasteurized milk belonged to the *Escherichia* section, 26 per cent to the *Aerobacter* section and 16 per cent to the intermediate section. There is no significant difference between the results obtained by the author and those reported by Kline.

Six species of the genus *Escherichia* were found (Table 2). *Escherichia coli* comprised 10 per cent of the cultures; *E. pseudocoloides* 24 per cent; *E. communior* 5 per cent; *E. paragrünthali* 10 per cent; *E. vesiculiformans* 5 per cent and *E. grünthali* 5 per cent.

Aerobacter cloacae comprised 10 per cent of the cultures and was the only species of the genus *Aerobacter*.

Raw Cream

Thirteen samples of sweet and 11 samples of sour raw cream were examined from cream supplied the Iowa State College dairy department for butter manufacture. Sixty per cent of the samples contained over 10,000 and 20 per cent over 100,000 *Escherichia-Aerobacter* organisms per cc. The number of *Escherichia-Aerobacter* organisms ranged between 25 per cc. and 250,000 per cc. Counts of the sweet cream were approximately the same as the counts of the sour cream.

Eighty-six per cent of the cultures were isolated by the direct plating method and 14 per cent by the enrichment method. Of 42 cultures isolated from 24 samples of cream 33 per cent belonged to the genus *Escherichia*, 57 per cent to the genus *Aerobacter* and 10 per cent to the intermediate group (Table 1).

Seven species of the genus *Escherichia* were found (Table 2). *Escherichia coli* comprised 10 per cent of the cultures; *E. communior* 7 per cent; *E. paragrünthali* 5 per cent; *E. formica* 5 per cent; *E. enterica* 2 per cent;

E. anaerogenes 2 per cent and *E. neapolitana* 2 per cent. Three species of the genus *Aerobacter* were found. *Aerobacter aerogenes* comprised 31 per cent of the cultures; *A. cloacae* 14 per cent and *A. oxytocum* 12 per cent.

Pasteurized Cream

Four batches of sweet cream supplied the Iowa State College dairy department for butter manufacture were pasteurized at 145° F. for 30 minutes. Samples were taken from the pasteurizing vat at the end of the holding period. Organisms belonging to the *Escherichia-Aerobacter* group were absent from 10 cc. quantities of the pasteurized cream.

Ice Cream

Twenty samples of ice cream from 11 commercial plants were studied. The number of *Escherichia-Aerobacter* organisms ranged between 2 per cc. and 2,500 per cc. Seventy per cent of the samples contained less than 100 *Escherichia-Aerobacter* organisms per cc. All of the cultures were isolated by the enrichment method. Of 16 cultures isolated from 20 samples of ice cream (Table 1), 31 per cent belonged to the genus *Escherichia*, 56 per cent to the genus *Aerobacter* and 13 per cent to the intermediate group.

Three species of the genus *Escherichia* were found (Table 2). *Escherichia coli* comprised 13 per cent of the cultures; *E. pseudocoloides* 6 per cent; and *E. communior* 13 per cent. Two species of the genus *Aerobacter* were found. *Aerobacter aerogenes* comprised 19 per cent of the cultures and *A. cloacae* 38 per cent.

Ropy Milk and Cream

Organisms of the *Escherichia-Aerobacter* group have frequently been reported as the cause of ropy milk and cream outbreaks which yearly cause considerable economic loss. Nine cultures were studied which were isolated by enrichment methods from six ropy raw milk and three ropy raw cream samples from commercial sources. *A. aerogenes* comprised 67 per cent of the cultures and *A. oxytocum* 33 per cent. (Table 2).

Defective Butter

Twenty-five cultures belonging to the *Escherichia-Aerobacter* group were studied which were isolated by Dr. B. W. Hammer and Dr. H. A. Derby from samples of defective butter. The cultures were isolated by an enrichment method in a search for other organisms. *A. aerogenes* was predominant, comprising 60 per cent of the cultures. *A. oxytocum* comprised 16 per cent of the cultures, *A. cloacae* 12 per cent and the intermediate group 12 per cent (Table 2). The absence of the *Escherichia* section indicates that conditions in butter are not favorable for growth or survival of these organisms at the temperatures at which butter is ordinarily held. The

presence of species belonging to the genus *Aerobacter* in defective butter indicates that they may be responsible for certain defects. Hammer and Yale (3) found that *Aerobacter* species regularly developed off-odors and flavors in either salted or unsalted butter held for 10 days at about 18° C., while *Escherichia* species did not.

Atypical Cultures

Cultures which could not be identified on the first attempt according to the scheme of classification used in Bergey's Manual were repurified and descriptive characters confirmed. Thirty-nine per cent of the total number of cultures (80 out of 204) could not then be identified.

Thirty-three per cent of the unidentified cultures belonged to the intermediate group by virtue of a positive or intermediate methyl red test accompanied by ability to utilize citrate as the sole source of carbon. The remaining cultures in this unidentified group differed from species described by Bergey by secondary characters which were not considered to be important enough to warrant the definition of new species. For the above reason, these cultures were considered atypical cultures of closely related species.

The number of typical and atypical cultures for each species are given in table 3. Data in table 3 show that 6 cultures of *E. pseudocoloides* and 1 culture of *E. vesiculiformans* were atypical in that no action on salicin was detected. One culture of *E. vesiculiformans* was atypical in that no action on dulcitol was observed. All 6 of the cultures identified as *E. formica* were atypical in that indol was formed. Twenty-one cultures of *A. aerogenes* (46 per cent of all *A. aerogenes* cultures) were atypical in that indol was formed. Ten cultures of *A. cloacae* (37 per cent of all *A. cloacae* cultures) were atypical in that gelatin liquefaction was not detected while one culture of *A. cloacae* was atypical in that no action on salicin was observed. Five cultures of *A. oxytocum* (29 per cent of all *A. oxytocum* cultures) were atypical in that indol was not formed.

In view of the large number of cultures found to be atypical with respect to indol formation, the constancy of this character was determined by repeating a study of 136 cultures comprising approximately equal numbers of indol positive and indol negative cultures. The second indol test which was made three months after the first test gave a perfect correlation with the results of the first test.

Ten cultures belonging to the genus *Aerobacter* were motile but did not liquefy gelatin. None of the species in the classification given in Bergey's Manual would correspond to this description. As gelatin liquefaction is frequently slow and relatively difficult to recognize (Weldin 17), it is probably a less reliable character than motility and for this reason, the cultures

TABLE 3

Typical and atypical cultures classified as species of the Escherichia-Aerobacter group

SPECIES	TYPICAL CULTURES	ATYPICAL CULTURES	ATYPICAL CHARACTER
E. coli	17	3	No action on salicin.
E. communior	14	0	
E. paragrünthali	12	0	
E. pseudocoloides	9	6	No action on salicin.
E. vesiculiformans	5	2	No action on salicin (1); no action on dulcitol (1).
E. formica	0	6	Indol formed
E. enterica	5	0	
E. anaerogenes	4	0	
E. grünthali	3	0	
E. neapolitana	2	0	
A. aerogenes	25	21	Indol formed
A. cloacae	16	11	No action on salicin (1); no liquefaction of gelatin (10).
A. oxytocum	12	5	Indol not formed.
Total	124	54	

were classified as atypical *A. cloacae,* atypical in that gelatin liquefaction was not observed.

DISCUSSION

The proportion of organisms belonging to each section of the *Escherichia-Aerobacter* group is chiefly determined by the source of contamination and by the temperature at which the dairy product is held. Inasmuch as dairy products vary widely in the above respects, the results obtained in this study might have been entirely different under other conditions. More species would undoubtedly have been isolated if a larger number of samples had been studied. However, the species which were found are undoubtedly the ones most commonly occurring in dairy products.

At the storage temperatures at which milk and cream are ordinarily held, 4.4°–10° C., species of the genus *Aerobacter* commonly grow more rapidly than do species of the genus *Escherichia.* The raw cream studied was held in storage for 24–72 hours before delivery while the raw and pasteurized milk was held for only 2–16 hours before delivery. The longer length of time during which the cream was held in storage was probably responsible for a greater proportion of the cultures belonging to the genus *Aerobacter* (57 per cent) than in the case of the raw and pasteurized milk (26 and 10 per cents).

The most common species in the dairy products studied were as follows: *Escherichia coli* in raw milk; *E. pseudocoloides* in pasteurized milk; *Aero-*

bacter cloacae in ice cream and *A. aerogenes* in raw cream, ropy milk and cream, and in defective butter. The predominance of these species in the different dairy products may be of more practical significance than is now realized.

SUMMARY AND CONCLUSIONS

1. Two hundred four cultures belonging to the *Escherichia-Aerobacter* group of bacteria were isolated from 212 samples of dairy products and were identified on a species basis according to the scheme of classification outlined in Bergey's Manual. Ninety-one cultures were isolated from 70 samples of raw milk; 21 cultures from 64 samples of pasteurized milk; 42 cultures from 24 samples of raw cream; 16 cultures from 20 samples of ice cream; 9 cultures from 9 samples of ropy milk and cream; and 25 cultures from 25 samples of defective butter.

2. The genus *Escherichia* comprised 63 per cent of the cultures from the raw milk; 57 per cent from pasteurized milk; 33 per cent from the raw cream; and 31 per cent from the ice cream.

3. The genus *Aerobacter* comprised 26 per cent of the cultures from the raw milk; 10 per cent from the pasteurized milk; 57 per cent from the raw cream; 56 per cent from the ice cream; 100 per cent from the ropy milk and cream, and 88 per cent from the defective butter.

4. The intermediate group comprised 11 per cent. of the cultures from the raw milk; 33 per cent from the pasteurized milk; 10 per cent from the raw cream; 13 per cent from the ice cream; and 12.0 per cent from the defective butter.

5. Ten of the 29 species listed by Bergey in the genus *Escherichia* were isolated from dairy products and identified as follows: *Escherichia coli, E. pseudocoloides, E. communior, E. paragrünthali, E. vesiculiformans, E. formica, E. enterica, E. anaerogenes, E. grünthali* and *E. neapolitana.*

Three of the 6 species listed by Bergey in the genus *Aerobacter* were isolated from dairy products and identified as follows: *Aerobacter aerogenes, A. cloacae* and *A. oxytocum.*

6. The most common species in the dairy products studied were *Escherichia coli* in the raw milk; *E. pseudocoloides* in pasteurized milk; *A. cloacae* in ice cream; and *A. aerogenes* in raw cream, ropy milk and cream, and defective butter.

7. Eighty of the 204 cultures could not be identified according to the scheme of classification used. Twenty-six of these unidentified cultures belonged to a group intermediate between the *Escherichia* and *Aerobacter* genera while the remaining unidentified cultures were classified as atypical cultures of various species.

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THE PRESERVATION OF SKIMMILK CURD FOR POULTRY FEEDING

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INTRODUCTION

A considerable number of the dairymen of the state do not have a profitable market for their skimmilk, while the poultrymen of the state are paying high prices for animal protein in the form of semi-solid buttermilk and other feeds. It is not practicable for the poultryman to make daily trips to the dairy. He needs considerably more milk at one season of the year than at another. He must have this milk at the time of greatest need and not when the dairyman happens to have it.

This skimmilk can be preserved in the form of curd. Removing a large part of the whey reduces the bulk and the curd which remains provides a concentrated protein feed of excellent quality.

The purpose of this investigation was to find a practical method for preserving skimmilk curd on the dairy farm in a satisfactory condition for poultry feeding and to determine the length of time the curd may be held.

Formaldehyde is very effective in preventing the growth of organisms in milk and other commodities. Chester and Brown (1) found that when the concentration of formaldehyde in milk was from 1:2,000 up to 1:800 there was a rapid decrease of bacteria during the first 24 hours, after which there was a slow decrease, until by the end of five days the milk was practically sterile, or contained only resistant spores. With milk containing 1:5,000 of formaldehyde there was a rapid reduction of bacteria during the first four to six hours, then a decrease after which multiplication began. A proportion of 1:10,000 gave, in some cases, a slight reduction before the increase started while 1:20,000 only slowed up the increase for a time. With milk containing 1:40,000 the increase was rapid from the first but the milk kept two to three times as long as untreated milk, and with 1:20,000 four times as long. Formaldehyde appeared to have less effect on *S. lactis* than on other bacteria commonly found in milk.

Price (2) found that formaldehyde in proportion of 1:20,000 prevents the development of the more common bacteria found in milk and in 1:1,560 it kills these bacteria. With milk containing 1:2,500 or less there was no effect on the activity of the fresh enzymes, rennet, pepsin, pancreatin, and steapsin *in vitro*. Formaldehyde may be added to milk in

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[1] The author acknowledges with thanks the valuable assistance rendered by F. W. Richardson, Superintendent of the University Poultry Farm, in supervising the feeding of the curd to poultry.

sufficient quantities to preserve the milk and prevent the development of some of the more common bacteria, that is, 1: 10,000 and still have no deleterious effect on the digestibility of the milk *in vitro*.

Doane and Price (3) state that two calves fed an extended period with milk preserved with formaldehyde made very satisfactory gains in weight and continued in perfect condition. They also state that pigs and children fed boric acid and formaldehyde at the instance of the British Commission suffered no evil result from the preservative and the pigs made substantial gains.

Scott (4) found that formaldehyde in dilutions of 0.5 to 0.7 per cent sterilizes anaerobic cultures rapidly.

The author found that formalin (40 per cent solution of formaldehyde) in proportion of 1: 500 prevented rennet from curdling milk while in 1: 600 the milk eventually curdled.

EXPERIMENTAL METHODS

Batches of curd were prepared by removing whey at the rate of about 60 per cent of the original milk. Preservatives were added and the curd was stored in containers for varying lengths of time.

Three different preservatives, viz., formalin (40 per cent solution of formaldehyde), benzoate of soda, and tri-sulpho-carbolate tablets were used. Curd was also stored without any preservative being added. Formalin and benzoate of soda were added to the curd in amounts ranging by volume from 1: 1,000 to 1: 250, while tri-sulpho-carbolate tablets were used in amounts ranging from 1 tablet to each gallon of curd to one and one-fourth tablet to each quart of curd.

Curd was stored in "shotgun cans" at different times of the year and held for periods ranging from 48 to 185 days. One batch of curd was stored in hermetically sealed glass fruit jars and held for 2 years and 4 months. Some of the curd was held in the laboratory where the temperature had a daily variation of about 62 to 70° F., during the winter months. During the spring and fall the temperature varied from about 68 to 75° F., while the temperature for the summer ranged from about 80 to 92° F. Most of the curd was stored in a boxed well house where the conditions would be similar to what one would expect to find on the average dairy farm. The temperature varied from a minimum of about 35° F. to a maximum of 95° F., during the year.

It was soon discovered that the curd containing benzoate of soda, and tri-sulpho-carbolate tablets, as well as that without any preservative would not keep in good condition for any length of time except during the winter. During the hot weather this curd soon digested. Therefore, after a few trials all methods were discontinued except treatment with formalin.

Preparing the Curd

1. Use good fresh skimmilk.

2. Bring the temperature of the milk to 68° F. to 72° F.

3. Add 1 to 2 quarts of good sour milk or good sour whey to each 10 gallons of milk. Stir thoroughly.

4. The addition of a small amount of rennet will facilitate the extraction of the whey, but it is not essential.

If rennet is used add one junket tablet to 30 or 40 gallons of milk. Dissolve the junket tablet in about one-half glass of cold water, add slowly and stir continuously for 3 or 4 minutes.

5. Let stand until a medium soft curd has formed. Cut into one-half inch cubes.

If the curd is too hard when cut it may be difficult to extract the whey.

6. Bring the temperature to 90° F. to 100° F. and let stand until the whey contains at least 0.7 per cent acid. It will take about 24 hours from the time of setting to develop sufficient acid.

7. Remove about 60 per cent of the milk in the form of whey—leave enough whey to cover the curd.

8. Add formalin (commercial formaldehyde) at the rate of 1: 1,000, *viz.*, 1.4 ounces of formalin to 10 gallons of curd. Mix formalin with curd.

9. Transfer curd immediately to container for storing and cover tightly.

10. Observe curd occasionally and if mold is forming add enough formalin solution (1: 1,000) to cover curd. Mold will not develop if curd is covered with moisture.

Containers

"Shotgun" or "Deep setting cans" were used for containers in the experimental work. Good tight wooden barrels or kegs should be satisfactory for use on the farm where large amounts of curd are to be preserved. Milk cans which have become rusty and therefore not satisfactory for handling milk and cream may be used for containers. Galvanized iron containers should not be used because of the danger of poisoning by the formation of zinc lactate. The containers should be promptly covered after each daily addition of curd and when finally filled a cover should be provided which is as nearly airtight as possible.

EXPERIMENTAL RESULTS

Eighteen cans of curd out of a total of 27, from 10 different batches with high acidity, kept in good condition while 5 cans were in poor condition and 4 were spoiled. Two of the spoiled cans were from gassy curd and one of the damaged cans was the result of a leak in the can. All the high acid curd which was kept covered with whey or formaldehyde solution kept in good condition.

The high acid curd was held for periods ranging from 112 to 185 days.

The low acid curd did not keep so well. Only two cans of curd out of a

total of seven, from three different batches with low acidity, kept in good condition while three were fair and two spoiled. The two cans which were good were held only 48 days. None of the sweet curd was held during the hottest weather.

Eleven of the 12 hermetically sealed jars of curd which were held for 2 years and 4 months kept in good condition. One sample spoiled because of a leak in the jar. The whey of six of these samples contained only 0.18 per cent acid while the whey of the other six had an acidity of 0.7 per cent at the time of adding the preservative.

The only visual difference in samples containing different amounts of formalin was discoloration of the whey and curd. There was a uniform increase in discoloration with the decrease in formalin. The jars containing formalin 1:1,000 showed considerably more discoloration than those with 1:500.

The curd was fed to poultry by the Poultry Husbandry Department and no bad effects were observed, even with young chicks. The curd was

TABLE 1

Keeping quality of curd held in "shotgun cans" for periods ranging from 112 to 185 days

AMOUNT OF FORMALIN	NUMBER OF SAMPLES	CONDITION OF SAMPLES			
		Good	Fair	Poor	Digested
		High Acid Curd			
1–250	4	3	0	1	0
1–500	10	7	0	1	2
1–750	2	1	0	1	0
1–1000	11	7	0	2	2
		Low Acid Curd			
1–1000	7	2*	3	0	2

* Held only 48 days.

TABLE 2

Effect of acidity on keeping quality of curd

AMOUNT OF ACID	NUMBER OF SAMPLES	CONDITION OF SAMPLES			
		Good	Fair	Poor	Digested
Less than 0.3%	7	2*	3	0	2
0.6 to .69%	5	4	0	0	1
0.7 to 0.76%	18	10	0	5	3
0.82%	4	4	0	0	0

* Held only 48 days.

relished by the birds of different ages although some of it was somewhat tough as a result of the effect of the formalin.

The available data on the action of weak solutions of formaldehyde on the digestive enzymes in acid solution would indicate that one should expect no bad results in feeding sour curd containing formalin 1: 1,000 as used in this experiment.

TABLE 3

Keeping quality of curd held in hermetically sealed glass jars for 2 years and 4 months

NO. OF JAR	ACIDITY OF WHEY, PER CENT	AMOUNT OF FORMALIN	CONDITION OF CURD WHEN FED
1	.18	1: 500	Good
2	"	1: 600	"
3	"	1: 700	"
4	"	1: 800	"
5	"	1: 900	"
6	"	1: 1000	Digesting
7	.70	1: 500	Good
8	"	1: 600	"
9	"	1: 700	"
10	"	1: 800	"
11	"	1: 900	"
12	"	1: 1000	"

CONCLUSIONS

Skimmilk curd can be preserved for 6 months or longer by the addition of formalin (40% formaldehyde) at the rate of 1: 1,000 provided the acidity of the whey is at least 0.7 per cent at the time of adding the formalin. This preserved curd makes a good animal protein feed for poultry.

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American Dairy Science Association Announcements

TENTH WORLD DAIRY CONGRESS

A news note from the U. S. Department of Agriculture states that O. E. Reed, "who was designated Official Correspondent for the United States at the Ninth congress in Denmark in 1931, has just received a communication from Secretary E. Hegh of the International Dairy Federation stating that the Tenth congress will be held at Rome and Milan, from April 30 to May 6. Secretary Hegh further instructed Mr. Reed to prepare a list of topics which he considers 'opportune for discussion' and to appoint speakers or reporters to present the material.

"A copy of the provisional program indicates that the congress will consider the reports and discussions from the various countries under seven major divisions: (1) Dairy-cattle breeding and milk production; (2) Sanitation, chemistry, and bacteriology of milk and its products; (3) Industry and commerce of milk and its products; (4) Educating the public to the value of milk; (5) Legislation and control of milk and its products; (6) Veterinary questions relative to milk; and (7) Dairying in the Tropics."

PUBLIC HEALTH AND INSPECTORS' MEETINGS

American Public Health Association will hold its annual meeting at the Hotel Claypool in Indianapolis on October 8 to 12, inclusive. For the complete program consult the September issue of the *American Journal of Public Health*.

International Association of Dairy and Milk Inspectors will meet in the same place on October 12 to 14, inclusive. The joint session on Thursday October 12 will be of special interest to dairy science and the dairy industry as special attention will be given to laboratory methods of milk analysis and the food value of dairy products.

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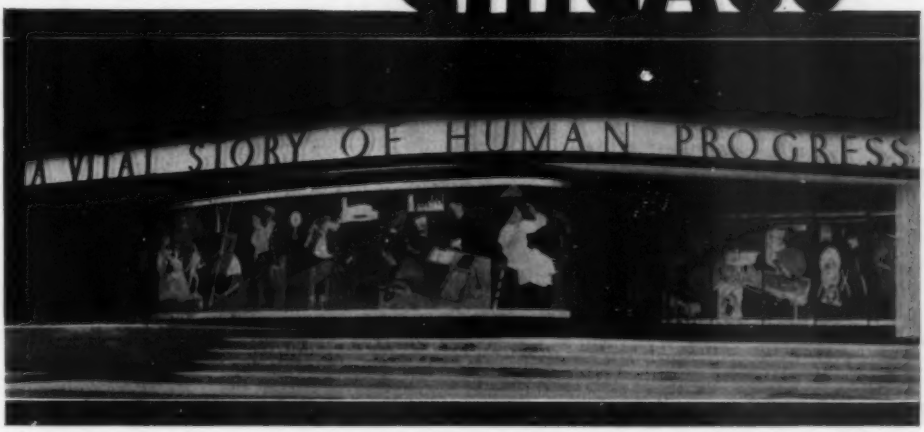

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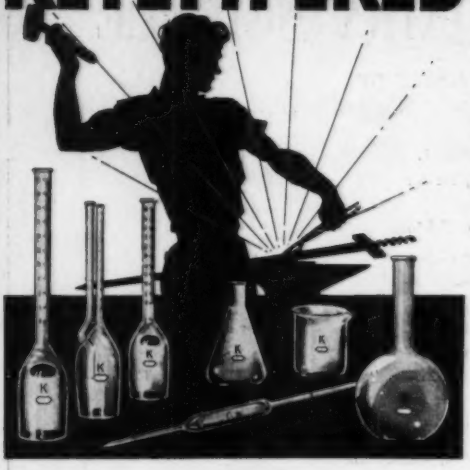

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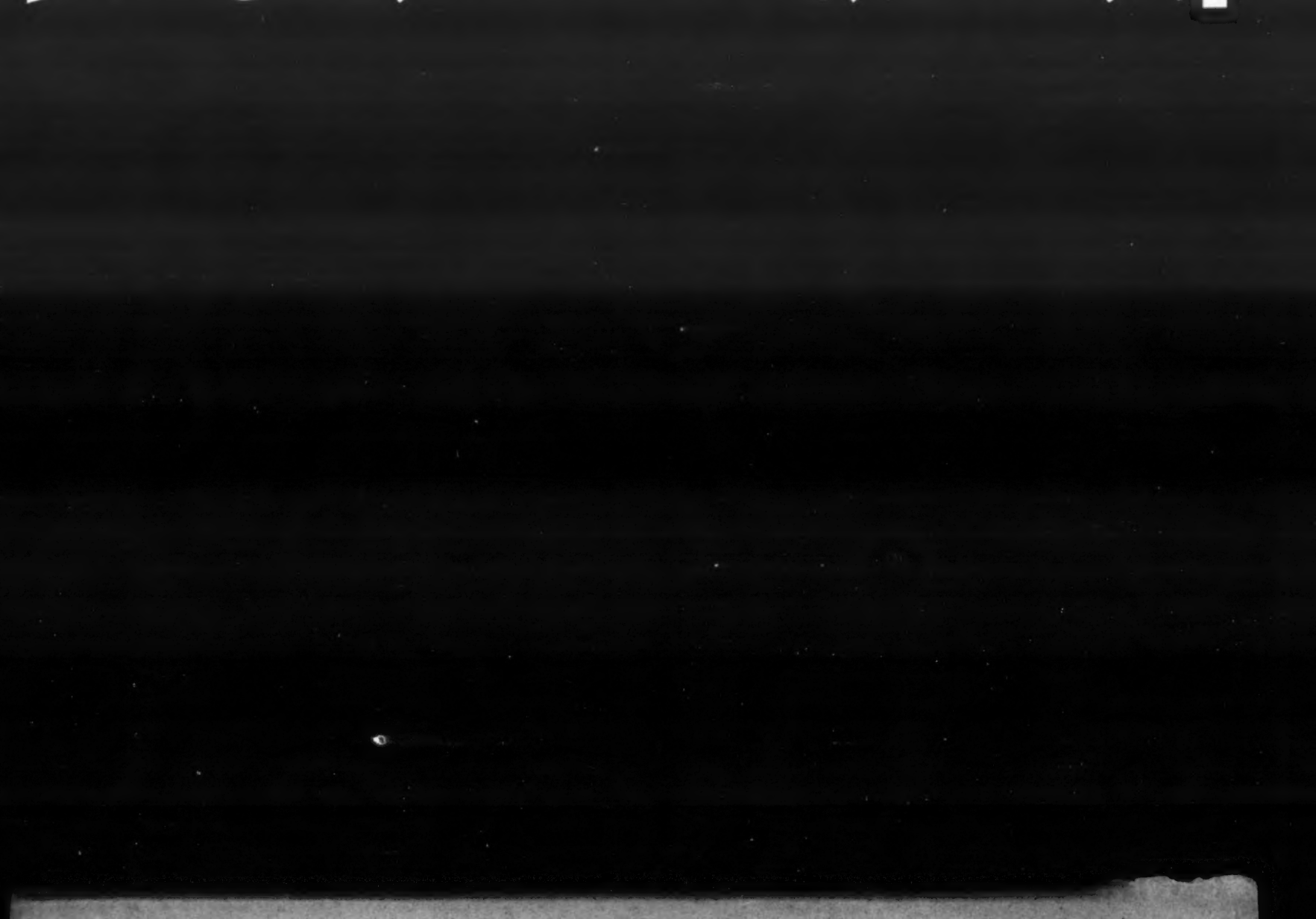
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